THE INFLUENCE OF DEXTRIN AND SUCROSE ON GROWTH AND DERMATITIS

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FIVE FIGURES

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In the study of the water-soluble vitamins of the B-complex the carbohydrate constituent of the basal ration appears to be a factor influencing both the incidence of dermatitis and rate of growth. The observations of Hogan and Richardson ('32 and '34), who report a high incidence of dermatitis in rats when using sucrose, and the more recent studies by Guerrant and Dutcher ('34) who observed a marked difference in rate of growth when using different carbohydrates, are particularly significant.

For comparative studies concerning the vitamin B-complex in milk and its derivatives and in rice polish, dextrin, as prepared in these laboratories, has been used as the carbohydrate of the basal rations. This product was made from a refined cornstarch, moistened with a 0.2 per cent citric acid solution, autoclaved for 5 hours at 120°C., and subsequently dried and ground. Various rations containing this dextrin have been used successfully with 2000 or 3000 white rats in comparative studies involving growth rate and polyneuritis. The 'pellagra-like condition' as described by Goldberger and Lillie ('26), the 'characteristic dermatitis' as observed by Hogan and Richardson ('32), the '(a) type of dermatis' as recorded by Chick, et al. ('35), and the 'pellagra-like dermatitis' as described by György ('35) has rarely if ever been noted.

However, in numerous cases a non-specific skin or fur condition, possibly indicative of an atypical dermatitis, has been observed especially in those animals the period of survival of which has been extended several weeks with test substances known to contain vitamin B(B_1) and possibly small amounts of the other factors of the vitamin B-complex.

The availability of pure vitamin B(B_1) (Merck) and pure lactoflavin as prepared in these laboratories (Supplee, et al., '36) will undoubtedly facilitate study of the vitamin B factors. The fruitfulness of such investigations however, may be predicated upon the carbohydrate employed in the basal ration. The influence of this constituent on the incidence of dermatitis and growth of white rats is shown by the following data wherein pure vitamin B and lactoflavin were used as the primary supplements.

EXPERIMENTAL

As a basis of departure, a basal ration was used containing dextrin, 62 parts; hydrogenated vegetable oil,[1] 10 parts; vitamin-free casein (Labco),[2] 20 parts; salt mixture 40,[3] 4 parts; powdered agar-agar, 2 parts; and cod liver oil, 2 parts. As the primary modification, the dextrin constituent of this ration was entirely replaced by sucrose; as a further modification the hydrogenated vegetable oil content was reduced from 10 to 3 parts and the sucrose content increased to 69 parts.

White rats, 22 to 26 days old and weighing 40 to 50 gm. when placed on the sucrose rations unsupplemented with other dietary factors showed little or no gain and survived for a period of only 2 to 5 weeks. Those on the dextrin rations gained from 2 to 5 gm. per week for a period of 5 to 8 weeks. No evidence of dermatitis has been observed from any of the unsupplemented rations, death occurring before the development of the

[1] Crisco.

[2] The Labco vitamin-free casein is distributed by The Casein Company of America, Inc., New York.

[3] H. Steenbock and E. M. Nelson. 1923. Fat-soluble vitamine. XIII. Light in its relation to ophthalmia and growth. J. Biol. Chem., vol. 56, p. 355.

symptoms. The survival period of the animals receiving the rations containing 10 parts of hydrogenated vegetable oil averaged from 1 to 2 weeks longer than those receiving the unsupplemented rations with the lower fat content.

Obviously a basal ration entirely devoid of the vitamins of the B-complex must be supplemented with one or more of the factors of this complex if a comprehensive experimental plan is to be carried out. To supply the antineuritic factor 2.5 γ or 12.5 γ of the crystalline vitamin B(B_1) (Merck) was fed as a daily supplement. When 12.5 γ supplemented the dextrin

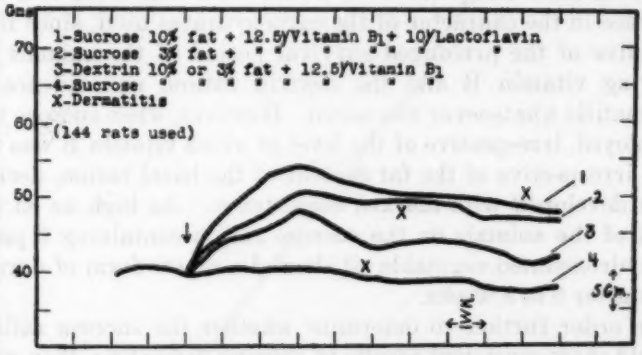

Fig. 1 Growth response of white rats on dextrin and sucrose rations supplemented with crystalline vitamin B, and crystalline lactoflavin.

rations some growth resulted for a short period of time, after which the weight remained stationary (fig. 1, curve 3); the survival period was extended over several months. No dermatitis of the character described by the investigators previously mentioned was observed. When the same amount of vitamin B supplement was fed with the sucrose rations containing either 10 or 3 parts of hydrogenated vegetable oil, the growth response was practically nil (fig. 1, curve 4). When 2.5 γ of vitamin B supplemented the sucrose rations the average survival period was shorter than for those animals receiving the same rations supplemented with 12.5 γ.

The significant reaction however, of almost all of the animals receiving the sucrose rations was the development of a dermatitis which is believed to be identical with that described by the various authors previously noted. This manifestation, illustrated in accompanying figure 4, was observed usually as early as the fifth week after starting the vitamin B supplement. The dermatitis also resulted from the sucrose rations with the higher amount of fat, but with less regularity than with the low fat sucrose ration; its appearance with the former was delayed on an average from 3 to 4 weeks.

From these comparisons it would seem that there is a difference in the character of the carbohydrates used, since irrespective of the prolonged survival period of the animals receiving vitamin B and the dextrin ration, no evidence of dermatitis whatsoever was noted. However, when sucrose was employed, irrespective of the level at which vitamin B was fed and irrespective of the fat content of the basal ration, dermatitis developed with marked consistency. As high as 85 per cent of the animals on the sucrose ration containing 3 parts of hydrogenated vegetable oil showed a severe form of dermatitis after 6 to 8 weeks.

In order further to determine whether the sucrose rations would show consistent results in causing dermatitis, they were supplemented with lactoflavin as well as with the pure vitamin B. The response of the animals receiving the low fat sucrose ration supplemented with 12.5 γ vitamin B and 10 γ of pure lactoflavin is shown in figure 1, curve 2, from which it will be noted that the incorporation of 10 γ of lactoflavin caused an immediate, although slight, growth response for a period of 2 to 3 weeks after which the weight remained practically stationary. The dermatitis developed after about 5 to 6 weeks with the same degree of regularity as when the same ration was supplemented with vitamin B only. When the same supplements were fed with the sucrose ration containing the higher amount of fat (fig. 1, curve 1) the incidence of the dermatitis was again delayed for a period of 3 to 4 weeks and its occurrence was not as regular as with the low fat ration.

A further comprehensive comparison of the dextrin and sucrose rations was made involving supplementation with the pure vitamin B, lactoflavin, and a lactoflavin-free material derived from a crude milk vitamin concentrate (Supplee, et al., '31). After an initial depletion period of about 2 weeks all

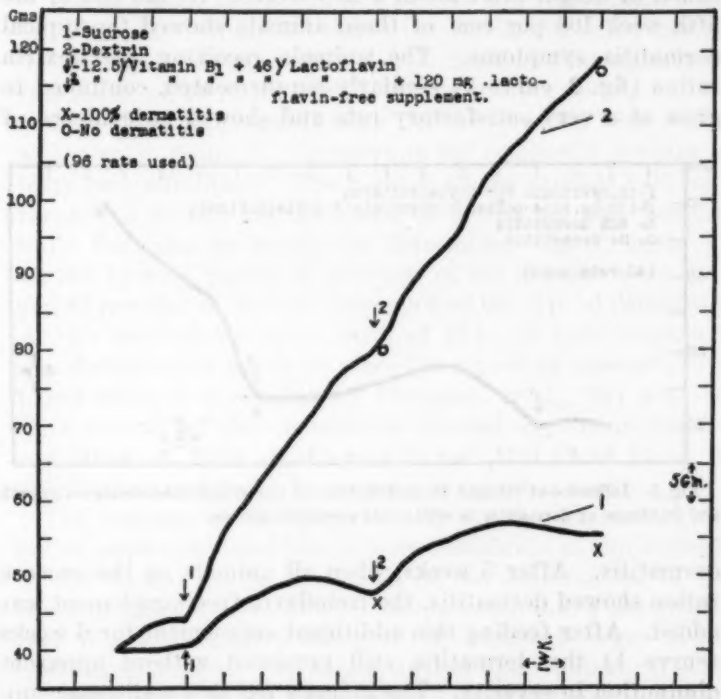

Fig. 2 Influence of dextrin and sucrose on growth and incidence of dermatitis in white rats receiving vitamin B_1 and lactoflavin supplements.

animals on the dextrin and low fat sucrose rations were fed a daily supplement consisting of 12.5 γ of pure crystalline vitamin B and 46 γ of lactoflavin contained in an impure concentrate (2-550) (Supplee, et al., '35). After a 5-week period of feeding these supplements each group was fed the crude milk vitamin concentrate previously freed from lactoflavin,

as an additional supplement in an amount equivalent to 120 mg. of the concentrate.

The animals receiving the sucrose ration (fig. 2, curve 1) supplemented with vitamin B and lactoflavin showed a definite growth response as a result of such supplementation, but declined in weight after about 3 to 4 weeks. At the end of the fifth week 100 per cent of these animals showed the typical dermatitis symptoms. The animals receiving the dextrin ration (fig. 2, curve 2) similarly supplemented, continued to grow at a very satisfactory rate and showed no evidence of

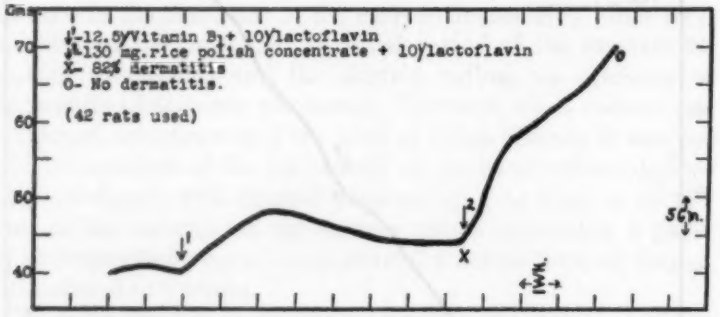

Fig. 3 Influence of vitamin B_1, lactoflavin and rice polish concentrate on growth and incidence of dermatitis in white rats receiving sucrose.

dermatitis. After 5 weeks, when all animals on the sucrose ration showed dermatitis, the lactoflavin-free supplement was added. After feeding this additional supplement for 6 weeks (curve 1) the dermatitis still persisted without apparent diminution in severity. The animals fed this additional supplement with the dextrin ration (curve 2) continued to grow and no evidence of dermatitis whatsoever was noted throughout the entire period of observation. These data seem to indicate that neither the vitamin B nor the lactoflavin, nor the lactoflavin-free material in the form and at the levels at which they were fed, had any influence on the occurrence of the dermatitis.

In order to determine whether the difference in reaction which resulted from the dextrin and sucrose rations may be due to a factor of vitamin character, or a condition in the ceca of the experimental animals (Guerrant, et al., '35) the following experiments were made.

The sucrose ration containing 3 parts of hydrogenated vegetable oil was employed. After the initial depletion period, the animals received vitamin B, lactoflavin and the lactoflavin-free material, all at the same levels as in the previous experiment. These supplements were fed for 7½ weeks with results as shown in figure 3; the curve is the composite average of forty-two individuals. The reaction of the animals both with respect to growth and incidence of dermatitis was substantially the same as previously determined (fig. 2, curve 1). At the 7½-week period 21 per cent of the animals were dead and 82 per cent of the survivors showed the typical dermatitis. At this juncture the supplement of 12.5 γ of pure vitamin B was discontinued and in its place 130 mg. of an especially prepared rice polish concentrate (Supplee, et al., '34) was fed. This amount of the concentrate carried approximately the equivalent of 12.5 γ of vitamin B, and also about 1 mg. of lipid matter and about 30 mg. of non-reducing sugars.

The response of the animals receiving this amount of rice polish concentrate and 10 γ of pure lactoflavin as supplements to the sucrose ration, is shown in the continuation of the curve in figure 3, from which it will be noted that a substantial rate of growth resulted and that the dermatitis began to improve immediately. The accompanying figure 5 illustrates the condition of the animals after about 1 week of feeding of these supplements. Definite signs of healing are clearly evident. After 4 weeks the dermatitis was cured and all the animals appeared to be normal in this respect.

It is evident that these results indicate the presence of an antidermatitis factor in the rice polish concentrate. Supplementary data showed that the small amounts of lipid matter and of non-reducing sugars carried by the concentrate are not the cause of the healing of the dermatitis observed in these

Fig. 4 Characteristic dermatitis resulting from the unsupplemented basal ration containing sucrose.

Fig. 5 Characteristic dermatitis resulting from the unsupplemented basal ration containing sucrose, cured by an aqueous extract of rice polish, 120 mg. daily.

experiments. Furthermore in view of the other experiments reported herein, it may be assumed that dextrin carries some of the antidermatitis factor, or that the favorable reaction of the dextrin fed rats is due to cecal fermentation as indicated by Guerrant, et al. ('35). The data are considered to be conclusive in showing that two of the entities of the vitamin B-complex, namely vitamin B and lactoflavin have no antidermatitis effect whatsoever when employed as supplements to the sucrose rations. Neither do these factors used singly or together permit normal or continued growth unless they are supplemented with a third factor or group of factors.

SUMMARY

1. Comparative data from basal rations containing dextrin and sucrose, respectively, show that no dermatitis resulted when the former was used, whereas a high incidence of dermatitis resulted when sucrose served as the basal carbohydrate.

2. Vitamin B and lactoflavin supplementing the sucrose ration did not prevent the development of dermatitis, nor did these supplements permit normal and continued growth; such supplements fed with dextrin promoted a substantial rate of growth.

3. A concentrate prepared from rice polish cured the dermatitis occurring in the sucrose fed animals and at the same time promoted a substantial rate of growth, provided adequate amounts of vitamin B and lactoflavin were fed simultaneously.

4. The occurrence of dermatitis was delayed and not as regular with a sucrose ration containing 10 per cent hydrogenated vegetable oil as with one containing 3 per cent of the same oil.

5. The data as a whole would seem to indicate that the basal ration containing 69 parts of sucrose and 3 parts of hydrogenated vegetable oil is well suited for the determination of the growth-promoting properties of lactoflavin, provided that it is supplemented with adequate amounts of vitamin B and the vitamin factor or group of factors contained in the rice polish concentrate.

ACKNOWLEDGMENT

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LACTOFLAVIN, A NECESSARY GROWTH-PROMOTING DIETARY FACTOR

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THREE FIGURES

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The current literature seems to show conclusively that lactoflavin irrespective of its source is a necessary growth-promoting dietary factor. However, the potency of the preparations of different workers varies considerably. Booher ('34) reports that 600 γ per rat per day induced a consistent and continuous growth rate of 5.0 to 6.0 ± 1.0 gm. per week over a 4-week period. Itter, et al. ('35) found that 100 γ produced an average weekly gain of 8 to 10 gm. for 3 weeks. Lepkovsky and collaborators ('35) state that 100 γ per day enabled rats to gain about 26 gm., and 300 γ about 30 gm. in 9 days. Recently Stare ('35) described a crystalline preparation of lactoflavin which gave an average weekly gain in weight of about 4 gm., when 50 γ was fed daily for about 9 weeks. The crystalline products prepared in Karrer's and Kuhn's laboratories are reported to have a considerably higher potency. According to Karrer, et al. ('35) a daily weight increase of 0.75 gm. is obtained, if 3 γ of the less easily soluble fraction of the crystalline lactoflavin is fed daily; v. Euler and co-workers ('34) report that 5 γ per rat per day produces an average daily gain of 1.1 gm. for 5 weeks and that 10 γ induces a daily gain in weight of 1.3 gm. for 5 weeks; Kuhn and his collaborators ('35) indicate that 10 γ of the natural lactoflavin produces a daily gain in weight of 1.45 gm. and 10 γ of the synthetic lactoflavin a gain of 1.43 gm. In view of these data it seems of

interest to report the results which have been obtained with the crystalline lactoflavin prepared in these laboratories (Supplee, et al., '36).

EXPERIMENTAL

The various data and observations recorded in a recent paper from these laboratories (Bender, et al., '36) showed that a sucrose containing basal ration entirely devoid of the vitamins of the B-complex, is well suited for the determination of the growth-promoting properties of lactoflavin, provided that it is supplemented with adequate amounts of vitamin $B(B_1)$ as well as with a third factor or group of factors contained in a specially prepared rice polish concentrate (Supplee, et al., '34). The basal ration consisting of sucrose, 69 parts; vitamin-free casein (Labco),[1] 20 parts; hydrogenated vegetable oil,[2] 3 parts; salt mixture 40,[3] 4 parts; powdered agar-agar, 2 parts; and cod liver oil, 2 parts, was used in the studies reported in the present paper, as this ration has been found to give reliable and consistent results, when fed to white rats, 22 to 26 days of age and weighing 40 to 50 gm.

After the depletion period the animals received various amounts of the rice polish concentrate as a daily supplement. This concentrate contains an insignificant amount, if any, of lactoflavin as determined by the previously described fluorometric method (Supplee, et al., '36) which is capable of detecting as little as 1 part in 20 million. The biological data which are presented in the following paragraphs substantiate the results obtained fluorometrically.

As a further supplement, 12.5 γ of the crystalline vitamin B (Merck) was fed. For determining the growth-promoting properties of lactoflavin, the pure crystalline product (Supplee, et al., '36) was fed simultaneously with either one

[1] The Labco vitamin-free casein is distributed by The Casein Company of America, Inc., New York.

[2] Crisco.

[3] H. Steenbock and E. M. Nelson. 1923. Fat-soluble vitamine. XIII. Light in its relation to ophthalmia and growth. J. Biol. Chem., vol. 56, p. 355.

or both of the above supplements in amounts varying between 2.0 and 20.0 γ.

The results from the feeding of the rice polish concentrate and lactoflavin are illustrated in figure 1, graphs 1 to 3. It is to

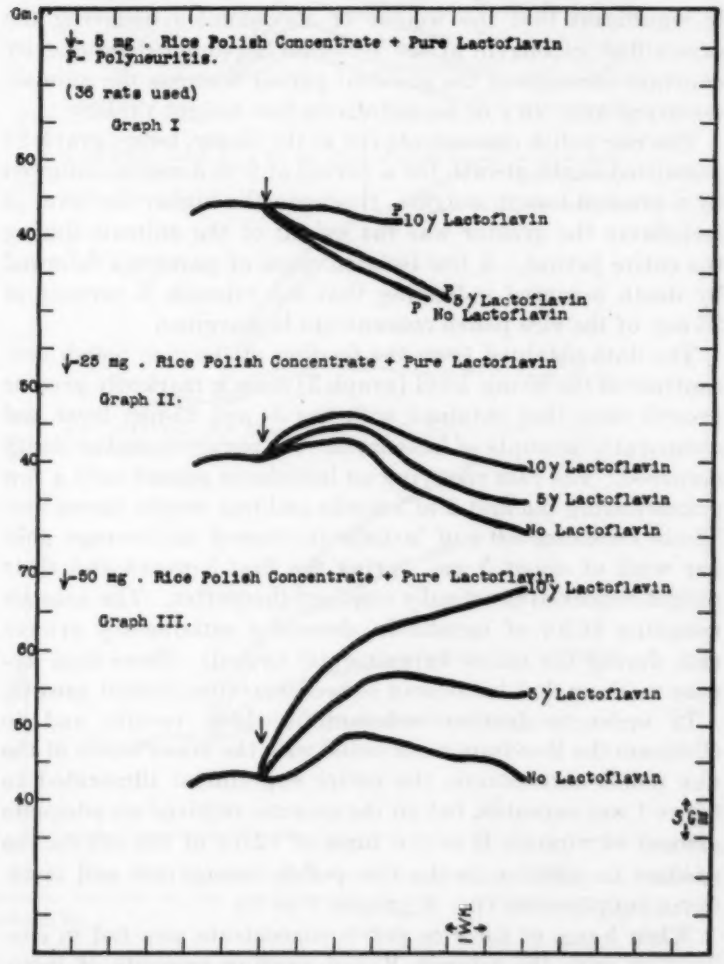

Fig. 1 The effect of pure lactoflavin on the growth of white rats receiving variable amounts of rice polish concentrate.

be noted that a relatively high percentage of the animals receiving the rice polish concentrate at the 5-mg. level (graph 1) developed paralysis and that all died within 4 to 5 weeks from B-avitaminosis, 5 mg. of the concentrate carrying only 0.48 γ of vitamin B as previous assays have shown. It is significant that the weight of the animals receiving the crystalline lactoflavin at the 10.0 γ level remained practically constant throughout the survival period whereas the animals receiving only 5.0 γ or no lactoflavin lost weight rapidly.

The rice polish concentrate fed at the 25-mg. level (graph 2) permitted slight growth for a period of 2 to 3 weeks, followed by a gradual loss in weight. However, the higher the level of lactoflavin the greater was the weight of the animals during the entire period. A few isolated cases of paralysis followed by death occurred indicating that the vitamin B content of 25 mg. of the rice polish concentrate is marginal.

The data obtained from the feeding of the rice polish concentrate at the 50-mg. level (graph 3) show a markedly greater growth than that obtained with the 5- and 25-mg. level and comparable amounts of lactoflavin. No paralysis and no death occurred. The rats receiving no lactoflavin gained only a few grams during the first 2 to 3 weeks and lost weight thereafter. Those receiving 5.0 γ of lactoflavin showed an average gain per week of about 5 gm. during the first 3 weeks and their weight remained practically constant thereafter. The animals receiving 10.0 γ of lactoflavin showed a satisfactory growth rate during the entire experimental period. These data appear to show that lactoflavin is necessary for normal growth.

In order to further substantiate these results and to eliminate the B-avitaminosis noted with the lower levels of the rice polish concentrate, the entire experiment illustrated in figure 1 was repeated, but all the animals received an adequate amount of vitamin B in the form of 12.5 γ of the crystalline product in addition to the rice polish concentrate and lactoflavin supplements (fig. 2, graphs 1 to 3).

When 5 mg. of the rice polish concentrate was fed in conjunction with the vitamin B and varying amounts of lactoflavin (graph 1), most animals survived, but showed the

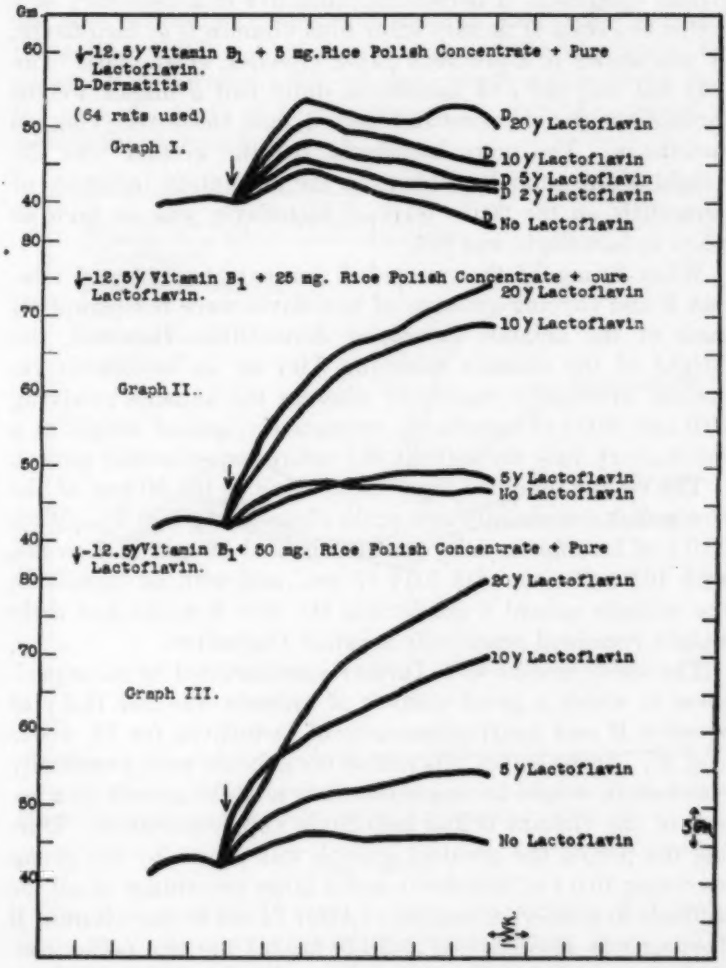

Fig. 2 The effect of pure lactoflavin on the growth of white rats receiving variable amounts of rice polish concentrate and a constant amount of pure vitamin B_1.

typical symptoms of dermatitis indicative of a deficiency in a factor or group of factors other than vitamin B or lactoflavin, as was shown in a previous paper (Bender, et al., '36). The rats fed only 2.0 γ of lactoflavin daily had a higher weight throughout the experimental period than those receiving no lactoflavin. The more lactoflavin fed the greater was the weight of the animals. However, the percentage incidence of dermatitis on the 20.0 γ level of lactoflavin was as high as when no lactoflavin was fed.

When 25 mg. of the rice polish concentrate, 12.5 γ of vitamin B and varying amounts of lactoflavin were fed (graph 2) none of the animals developed dermatitis. However, the weight of the animals receiving 5.0 γ or no lactoflavin remained practically stationary whereas the animals receiving 10.0 and 20.0 γ of lactoflavin, respectively, gained weight at a satisfactory rate throughout the entire experimental period.

The results obtained from the feeding of the 50 mg. of the rice polish concentrate are again clear-cut (graph 3). With 20.0 γ of lactoflavin daily the rats gained 38 gm. in 7 weeks, with 10.0 γ 28 gm., with 5.0 γ 13 gm., and with no lactoflavin the animals gained 4 gm. within the first 2 weeks and their weight remained practically constant thereafter.

The above results were further substantiated by an experiment in which a great number of animals was fed 12.5 γ of vitamin B and varying amounts of lactoflavin for 7½ weeks (fig. 3). At the end of this period the animals were practically constant in weight having shown only a slight growth as a result of the vitamin B and lactoflavin supplementation. During the period the greatest growth was shown by the group receiving 10.0 γ of lactoflavin and a large percentage of all the animals developed dermatitis. After 7½ weeks the vitamin B feeding was discontinued and 130 mg. of the rice polish concentrate was fed daily in its place. The response resulting from this supplement feeding is seen in the continuation of the curves in figure 3. The dermatitis was cured in all the animals. With 10.0 γ of lactoflavin a gain in weight of 36 gm. was obtained in 4 weeks, with 5.0 γ 20 gm., with 2.0 γ 12 gm.,

and with no lactoflavin the animals gained only 5 gm. during the first week, their weight remaining practically constant thereafter. This set of experiments emphasizes again the necessity of lactoflavin for normal growth and confirms that the rate of growth of rats is dependent upon the amount of lactoflavin in their feed.

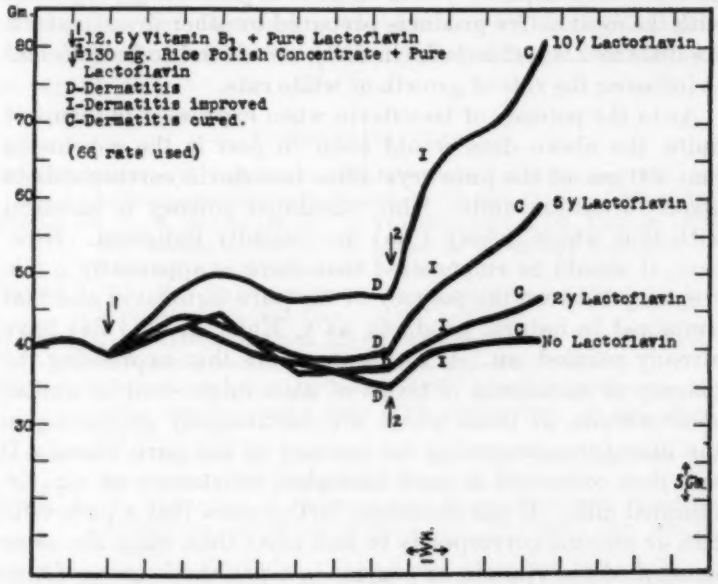

Fig. 3 The influence of vitamin B_1, rice polish concentrate and variable amounts of pure lactoflavin on growth and incidence of dermatitis in white rats.

DISCUSSION

The data as a whole seem to show conclusively that lactoflavin is a necessary growth-promoting dietary factor. The various results obtained point to the complexity of the biological assays for the various vitamins of the B-group. The vitamin B appears to be a prerequisite of such assays; a supplement feeding of 12.5 γ of the pure crystalline product which according to the manufacturer's data corresponds to 5 units of vitamin B, provides adequate amounts of the antineuritic

factor. The factor or group of factors which prevents and cures dermatitis and which is carried by the rice polish concentrate employed must likewise be supplied in adequate amounts, if the true growth-promoting properties of lactoflavin are to be determined.

Under such conditions the lactoflavin prepared in these laboratories compares well in its growth-promoting properties with the most active products prepared by other investigators. As little as 2.0 γ of lactoflavin daily was found to be sufficient to influence the rate of growth of white rats.

As to the potency of lactoflavin when expressed in terms of units, the above data would seem to permit the conclusion that 100 gm. of the pure crystalline lactoflavin corresponds to about 15 million units. This calculated potency is identical with that which Scheer ('35) has recently indicated. However, it should be emphasized that there is apparently a discrepancy between the potency of the pure lactoflavin and that contained in natural products, as v. Euler et al. ('34) have already pointed out. It seems therefore that expressing the potency of lactoflavin in terms of units might lead to similar controversies as those which are continuously appearing in the literature concerning the potency of the pure vitamin D and that contained in such biological substances as e.g., irradiated milk. If one considers furthermore that a pure vitamin or enzyme corresponds to less units than when the same amount of the vitamin or enzyme is a prosthetic group (Ansbacher and Supplee, '34) in a symplex (Willstätter and Rohdewald, '34), it becomes very doubtful whether the potency of biological substances such as vitamins and enzymes, can be expressed in units as determined by empirical procedures.

SUMMARY

Lactoflavin is a necessary growth-promoting dietary factor. A difference in the rate of growth of white rats results from the differences in the daily intake level of pure crystalline lactoflavin varying from 2.0 to 20.0 γ.

The growth-promoting properties of lactoflavin are readily determined with a suitable basal ration adequately supplemented with pure vitamin B and a third factor or group of factors necessary for the prevention and cure of dermatitis and carried by rice polish.

The potency of lactoflavin may be 'calculated' to be 150,000 units per gram. However, as briefly discussed, the 'unit' designation for expressing the potency of biological substances, such as vitamins and enzymes, may be meaningless or erroneous.

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LESIONS OF THE NERVOUS SYSTEM IN VITAMIN DEFICIENCY

IV. THE EFFECT OF CAROTENE IN THE TREATMENT OF THE NERVOUS DISORDER IN RATS FED A DIET LOW IN VITAMIN A

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TWO FIGURES

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In a previous communication (Zimmerman, '33) which detailed the lesions in the nervous systems of albino rats fed a diet low in vitamin A, it was shown that the animals developed muscular weakness and incoordination after a prolonged subsistence on the diet and after other signs of the deficiency, such as loss of weight and xerophthalamia, supervened. It was further shown that the neurologic manifestations were associated with degeneration of the medullary sheaths of the peripheral nerves and of the spinal cord. In every instance the animals displaying these nervous disorders were necropsied shortly after the appearance of the latter and it seemed worthwhile, therefore, to study in a new experiment the anatomic lesions at various periods in the recovery from these nervous manifestations. With the availability of carotene as a source of vitamin A, it was felt that this ideal could be realized. The following experiments were in part devised to test the effectiveness of carotene in preventing, alleviating or curing these manifestations. Moreover, it was hoped that

[1] The expenses of the study were defrayed in part by a grant from the Research Fund of the Yale University School of Medicine.

if carotene alone proved effective, an answer would be at hand to the question of the role which an absence of unsaturated fatty acids in the diet plays in the production of these nervous lesions.

Shortly after the appearance of the previous paper, some doubt was cast by Grinker and Kandel ('33) on the relation of vitamin A-deficiency to changes in the nervous system. The present communication also deals in part, therefore, with experiments devised to test the reproducibility of the results previously reported.

EXPERIMENTAL PROCEDURE

A total of forty-three albino rats, the offspring of carefully selected and dieted animals maintained in the laboratory of physiological chemistry for breeding purposes, was employed in this study. These animals received the artificial ration low in vitamin A after weaning, which occurred between 21 and 24 days of age. The rats employed as controls received daily, in addition to this ration, 2 drops of a 0.1 per cent solution of carotene in cottonseed oil; one drop of this solution was equivalent to 25 micrograms (25 γ). A similar amount of carotene and sometimes, as will be specified below, double this amount was employed in the 'treatment' experiments.

Diet employed. The composition of the artificial ration low in vitamin A was:

	per cent
Casein (vitamin-free)[1]	18
Corn starch	57.8
Hydrogenated vegetable oil[2]	20
Osborne-Mendel salt mixture IV	4
Linoleic acid	0.2

Linoleic acid was added to the ration in order to supply an adequate amount of unsaturated fatty acid. The hydrogenated vegetable oil used is known to contain such acids, but since their amount may vary from time to time it seemed advisable to incorporate in the ration an adequate supply in the form of linoleic acid.

[1] From the Harris Laboratories, Tuckahoe, New York.
[2] Crisco.

The vitamin B complex was supplied to all rats daily in the form of 0.3 gm. of dried yeast.[4] Vitamin D was supplied in the form of a solution of irradiated ergosterol in cottonseed oil, 17 International Units being supplied daily. Vitamin E is present in sufficient quantity in the vegetable oil; vitamin C is apparently not needed by the rat.

Grouping of animals. The rats were grouped into five classes in accordance with experimental conditions specified for each group.

Group I was composed of nine animals which were maintained on the artificial, vitamin A-low diet from weaning until the end of the experiment. At no time did these animals receive any carotene or other source of vitamin A. The purpose of this experiment was simply to determine whether the results described in the previous communication (Zimmerman, '33) were reproducible.

Group II comprised a total of fifteen animals which were maintained on the same artificial ration low in vitamin A, but which were treated with carotene when marked loss of weight, xerophthalmia and often also 'paralysis' supervened. On the whole, these animals were ineffectively, and probably also inadequately, treated as will be brought out below. In each instance death ensued spontaneously, with but one exception, within 1 to 5 days after the beginning and in spite of therapy. This group in reality represents an 'accident' in this study, for at the beginning of the experiment the intention was to treat the animals adequately. Either because therapy was instituted too late in the course or because insufficient dosage was employed, the animals failed to survive.

Group III consisted of six rats which were effectively treated with carotene after developing loss of weight, xerophthalmia and frequently also 'paralysis,' when fed the artificial diet low in vitamin A. This group differs from group II in that each of these animals began to increase in weight, had a restitution to normal of its ophthalmic condition and in some instances showed an amelioration of its neurologic condition

[4] From the Northwestern Yeast Company, Chicago, Illinois.

as judged by its behavior. In each instance the animals belonging to this group were killed with ether after a prolonged period of recovery sufficient to indicate the effectiveness of carotene as a therapeutic agent in this condition.

Group IV was composed of seven animals fed the artificial ration and 2 drops of a 0.1 per cent solution of carotene in cottonseed oil from weaning until the termination of the study. These animals constituted the positive controls. One animal from each litter was placed in this group; the remainder of the litter mates were scattered among the other groups.

Group V was composed of six rats which were originally started on the vitamin A-low diet but were soon, at various times for each animal, given the basal dosage (2 drops daily of carotene) and were thereby converted into control animals. The reason for this change differed for each animal, but was usually due to the fact that the animal ate poorly and lost weight too rapidly or refused to eat the allotted yeast and thereby may have developed a complicated deficiency or showed other complicating features, such as, unusual weakness and early infection of the urinary tract.

Neurological manifestations. During the progress of these experiments the animals were examined twice weekly until the first sign of a change in their condition, when examinations were made daily or on alternate days. At such times accurate record was made of the body weight, amount of food and vitamin consumption and general physical condition. Special attention was paid to evidence of xerophthalmia and neurologic manifestations. A few simple tests were employed to help in the study of the nervous changes, and these were as follows:

1. Observing the gait on a smooth, level surface, noting especially any tendency to drag the posterior extremities.

2. Holding the animal in a vertical position by the skin of the neck and back to note the posture of the posterior extremities. Paralyzed or weak extremities are hyperextended whereas normal extremities are flexed. This was previously described by Aberle ('34).

3. Climbing over obstacles.

4. Maintaining position on a rotating, loose-meshed wire screen. Records of the performance in these tests were made with the cinema for the comparison of each animal's ability at different times and also with that of the control animals. Occasionally, in spite of these tests, it was difficult to determine whether the neurologic manifestations were simply weakness, muscular incoordination, paresis or actual paralysis. For the sake of brevity in the following tables, all of these neurologic changes were designated by the term 'paralysis,' even if this term was not strictly descriptive of the true condition in a particular instance. The degree of severity of 'paralysis' was designated as moderate (+) or marked (++); weakness was usually designated by the symbol ±.

Material studied and technic employed. Both sciatic nerves, the brachial plexuses, the entire spinal cord and the cerebrum of each animal were removed at necropsy and fixed at once in the proper fixatives. The postmortem examinations were performed immediately after the animals were killed with ether; when the animals died spontaneously the examinations were often unavoidably delayed for 1 hour, but in no instance for more than 2 hours.

The fixatives employed and the methods of fixation of the tissues were the same as those described in the previous communication (Zimmerman, '33). The tissues were sectioned as before and were stained by the Nissl, Marchi, Spielmeyer and Kulschitzky methods and with Sudan III.

RESULTS

Macroscopic examination of the brains, spinal cords and peripheral nerves of all the animals failed to reveal any abnormalities.

In none of the animals were any lesions demonstrable in the cerebrum by any of the staining methods. In every instance was the degree of myelin degeneration in the sciatic nerves more marked than in the brachial plexuses.

Group I. Nine rats fed diet low in vitamin A from beginning until termination of experiment. The essential data as concerns the duration of subsistence of each animal on the diet, the weight curve, the clinical manifestations of vitamin A-deficiency and the extent of the anatomic lesions produced thereby, are summarized in table 1.

All the animals in this group except rats 37 and 45 developed xerophthalmia as evidence of their vitamin A-deficiency. Two (rats 2 and 16) showed clinical signs of 'paralysis' of moderate degree, and two other animals (18 and 24) showed signs of muscular weakness. In the former two there was moderate

TABLE 1

Nine rats fed diet low in vitamin A

ANIMAL NUMBER	DURATION IN DAYS OF EXPERIMENT	WEIGHT IN GRAMS			XEROPHTHALMIA	'PARALYSIS'	DEATH	LESIONS	
		Initial	Maximum	Terminal				Peripheral nerves	Spinal Cord
2	59	70	138	98	+	+	Spontaneous	+	++
8	78	60	150	92	+	—	Spontaneous	+	+
9	64	58	120	106	+	—	Spontaneous	±	±
16	50	50	96	72	+	+	Spontaneous	+	++
17	59	58	106	98	+	—	Spontaneous	+	+
18	55	60	119	98	+	±	Spontaneous	+	+
24	94	53	192	116	+	±	Ether	+	+
37	43	45	98	72	—	—	Spontaneous	—	—
45	62	40	120	120	—	—	Ether	±	±

demyelination of the peripheral nerves and marked degeneration of the medullary sheaths in all the tracts of the spinal cord (fig. 1). In the latter two animals, both the peripheral nerves and the spinal cords showed a moderate degree of degeneration of the medullary sheaths. Again, two animals (8 and 17) which did not show any clinical evidence of neuromuscular disturbance showed moderate demyelination of the peripheral nerves and spinal cords. Rats 9 and 45 which had no clinical signs of nervous disease revealed equivocal changes (±) in their nervous systems. Animal 37, which probably had no vitamin deficiency although subsisting on the deficient ration for 43 days, showed neither clinical nor anatomic evi-

dence of nervous disease; this might be taken as suggesting that a period of 43 days under the conditions of these experiments is not long enough for the production of obvious specific signs of vitamin A-deficiency.

Group II. Fifteen rats fed diet low in vitamin A. Inadequate treatment with carotene. The animals in this group,

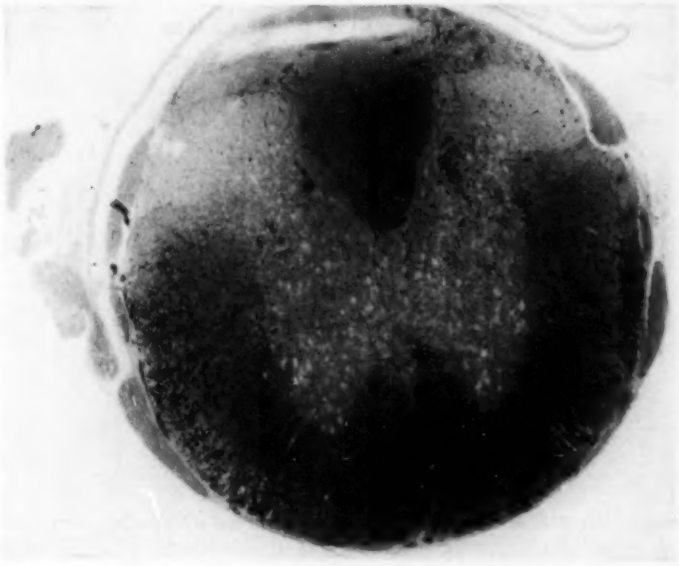

Fig. 1 Rat 16. Thoracic portion of spinal cord showing marked degeneration (black granules) of medullary sheaths in sensory and motor tracts. Marchi stain. × 12.

whose pertinent data are summarized in table 2, all developed xerophthalmia. Five of them (rats 3, 15, 23, 47 and 49) also showed definite signs of 'paralysis' and a sixth one (rat 27) had neuromuscular weakness. The animals without neurologic manifestations all showed a definite loss of weight which in some instances was alarming. In every instance the severity of the symptoms was the deciding factor in the institution of treatment with carotene, which was given in daily

dosage of 2 drops of a 0.1 per cent solution in cottonseed oil. The ineffectiveness of this therapy can be seen from the fact that all but one animal (rat 23) died spontaneously in from 1 to 5 days.

All the animals showed some degree of anatomic change in their nervous systems, which was marked (++) in three of them. The lesions, as in group I, consisted of demyelination of the sciatic nerves, brachial plexuses and spinal cords.

Fig. 2 Rat 51. Lower thoracic portion of spinal cord showing severe grade of degeneration of medullary sheaths (gray zone on periphery). Kulschitzky stain. × 10.

There was no evidence of a preferential or selective involvement of any one of the spinal fiber tracts.

Group III. Six rats fed diet low in vitamin A. Adequate treatment with carotene. Shortly after these animals developed clinical signs of vitamin A-deficiency as characterized by xerophthalmia, and before marked loss of weight ensued, they were started on carotene. The animals which showed signs of weakness or paralysis were given twice the daily dosage (4 drops of a 0.1 per cent solution or 100 micrograms). The animals which failed to show rapid signs of improvement

with the usual dosage of 2 drops were also given the larger amount, and thus all the animals in this group received 4 drops of carotene daily. Reference to table 3 reveals the fact that none of the animals died spontaneously. In every instance the course was terminated after the xerophthalmia was cured and the animal had made a remarkable gain in weight. In every case, also, the animal could obviously have

TABLE 2

Inadequate treatment of vitamin A-deficiency with carotene

ANIMAL NUMBER	DURATION IN DAYS OF EXPERIMENT	WEIGHT IN GRAMS			XEROPHTHALMIA	'PARALYSIS'	TREATMENT (DAYS)	DEATH	LESIONS	
		Initial	Maximum	Terminal					Peripheral nerves	Spinal cord
3	60	68	134	86	+	+	1	Spontaneous	—	+
5	54	70	142	122	+	—	1	Spontaneous	+	+
7	61	60	152	114	+	—	2	Spontaneous	+	±
11	61	64	134	88	+	—	2	Spontaneous	+	±
12	72	62	131	86	+	—	2	Spontaneous	+	++
14	60	58	112	86	+	—	1	Spontaneous	+	+
15	52	52	92	70	+	+	1	Spontaneous	±	+
23	82	58	198	116	+	+	1	Ether	±	+
27	84	58	212	134	+	±	1	Spontaneous	++	++
31	52	51	96	62	+	—	1	Spontaneous	—	+
32	45	52	101	94	+	—	1	Spontaneous	±	+
41	47	46	98	91	+	—	3	Spontaneous	+	+
42	51	40	94	80	+	—	3	Spontaneous	±	+
47	40	33	88	88	+	+	5	Spontaneous	++	++
49	40	32	92	84	+	+	1	Spontaneous	+	+

lived on indefinitely as far as its previous vitamin A-deficiency was concerned, were the experiment not arbitrarily terminated.

In table 3 the 'maximum weight' is that which the animal achieved while still being fed the deficient ration. It will be noted that animals 48, 50, 51 and 52 made remarkable gains which are listed under 'terminal weight.' Even animals 10 and 29, which were not given carotene over as long a period of time as the others, were well on the up-grade in their weight curve at the termination of the experiment.

In spite of these signs of improvement, the neurologic manifestations remained unimproved throughout the course. Indeed, the clearest picture of the paralysis was obtained after the xerophthalmia had cleared and the animals began to gain weight. The severest grade of degeneration of the nervous system in any of the experimental groups was noted anatomically also in these animals. Degeneration of the medullary sheaths was seen with equal clarity in the Marchi, Sudan III, Spielmeyer and Kulschitzky preparations (fig. 2).

Group IV. Seven control animals These rats, which received the standard artificial ration deficient in vitamin A and

TABLE 9

Adequate treatment of vitamin A-deficiency with carotene

ANIMAL NUMBER	DURATION IN DAYS OF EXPERIMENT	WEIGHT IN GRAMS			XEROPHTHALMIA	'PARALYSIS'	TREATMENT (DAYS)	DEATH	LESIONS	
		Initial	Maximum	Terminal					Peripheral nerves	Spinal cord
10	75	64	150	92	+	—	12	Ether	++	++
29	49	51	110	104	+	—	4	Ether	+	++
48	95	30	82	194	+	+	30	Ether	++	++
50	95	28	88	206	+	+	29	Ether	++	++
51	95	35	104	246	+	+	29	Ether	++	++
52	95	33	97	182	+	+	31	Ether	++	++

supplemented with 2 drops of carotene daily, subsisted on this regimen from 95 to 178 days. They gained in weight steadily from the beginning to the termination of the experiment. None of them developed xerophthalmia and none showed any clinical evidence of neurologic change. Microscopically their nervous systems were entirely normal.

Group V. Six rats converted into control animals late in experiment. For reasons already specified under 'experimental procedure,' these animals were converted into controls with a daily dosage of two drops of carotene after they had received the vitamin A-low diet for various periods of time. Each was killed with ether at the termination of the experiment which lasted from 101 to 170 days. One animal

(rat 26) was started on carotene before any signs of vitamin A-deficiency, including xerophthalmia, had developed. At the first signs of ocular abnormality, the remaining five animals (rats 25, 30, 40, 43 and 44) were started on a daily course of carotene administration. By that time, however, rat 40 had shown some weakness of its posterior extremities. Only two of the animals, rats 26 and 44, showed very slight anatomic changes of demyelination in their peripheral nerves and spinal cords. These changes were, indeed, so slight as to be of questionable significance. The remaining four animals had intact nervous systems on microscopic examination.

COMMENT

The results of the experiment with the animals in group I confirm the findings reported in the previous paper (Zimmerman, '33); namely, that rats subsisting on an artificial ration low in vitamin A often develop neurologic manifestations of disease which are due to demyelination of the peripheral nerves and spinal cord. These manifestations develop some time after the appearance of xerophthalmia, which is characteristic of vitamin A-deficiency, and in general there is a parallel between their severity and the degree of anatomic change in the nervous system. There is a certain amount of individual variation in these animals as regards the time of onset of the symptoms and the survival period. When untreated, however, these animals invariably succumb to the deficiency. Even those rats which succumb prior to the onset of any nervous symptoms show some degree of anatomic change in their nerves and spinal cords. It is quite conceivable that the clinical expression of these lesions is prevented in such instances by the intervening death of the animals.

What has just been stated concerning the results with the animals in group I applies equally to the results obtained with the animals in group II.

The experience with carotene as a therapeutic measure for the vitamin A-deficiency produced in this group of animals seemed disappointing. Its only effect was a possible prolonga-

tion of life for a day or two, but even this is questionable. Since it was realized that the animals were being treated late in their course, it was resolved to institute therapy earlier and to employ larger dosages in the experiment with group III. Here the effectiveness of carotene was shown beyond reasonable doubt. All the animals made remarkable improvement in their condition except for their neurologic symptoms.

The lesions in the nervous system were most severe in this group of animals, but this after all is not surprising in view of the fact that the nervous system has such poor ability to regenerate. The injury produced in it during the regime on the vitamin A-low diet is a permanent one and possibly even progressive. Thus the hope to be able to treat effectively the nervous disorder with carotene did not materialize, but an opportunity was provided to study its anatomic counterpart at various periods in the recovery from this vitamin deficiency.

In the experiment with group IV it was shown that carotene, as the only source of vitamin A, is effective in preventing all the symptoms and signs of this vitamin deficiency. Furthermore, the results of the experiment with group V indicate that carotene is equally effective in the prevention of nervous disorders in animals which have been deprived of this vitamin for various short periods of time.

Finally, these experiments as a whole show conclusively that the lesions in the nervous systems of animals subsisting on this artificial ration low in vitamin A are not dependent on an absence of unsaturated fatty acids. The presence of linoleic acid in the diet does not prevent these lesions from occurring, whereas the administration of carotene does accomplish this end.

CONCLUSIONS

1. Albino rats subsisting on an artificial ration adequate in all dietary essentials as far as is known except vitamin A develop a nervous disorder which is characterized by muscular weakness, incoordination and in the severest cases, paralysis of the posterior extremities.

2. The anatomic counterpart of this disorder is a demyelination of the peripheral nerves, most extensive in the sciatics, and of scattered fiber tracts in the spinal cord.

3. Carotene, when administered late in the course of this dietary deficiency or in inadequate (50 micrograms) dosage, does not alter the fatal outcome in any appreciable manner.

4. When administered relatively early in the course of the deficiency, and especially in adequate (100 micrograms) dosage, carotene is effective in restoring these animals to good health, the neurologic disorder alone persisting.

5. Animals thus treated with carotene resume their normal rate of growth and recover from the xerophthalmia, but their anatomic changes in the nervous system are even more marked than in the untreated animals. This is attributed to the relative inability of the nervous system to regenerate when once severely injured.

6. All evidence of this dietary deficiency is lacking in animals which receive the same artificial ration when the latter is supplemented with carotene (50 micrograms daily) throughout the experiment.

7. This study indicates that the changes in the nervous systems of animals fed the artificial diet low in vitamin A are due to a deficiency of this factor and not of unsaturated fatty acids.

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THE EFFECTS OF BREED CHARACTERISTICS AND STAGES OF LACTATION ON THE VITAMIN C (ASCORBIC ACID) CONTENT OF COW'S MILK [1]

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ONE FIGURE

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While milk is not usually considered a vitamin C rich food, from the standpoint of infant feeding it does constitute an important source of this dietary essential. In fact Hess ('20) has reported that one pint of fresh raw milk per day furnishes sufficient vitamin C for the average infant. Chick, Hume and Skelton ('20) found, however, that about 85 ml. of milk were required to furnish a minimum protective dose of vitamin C for the guinea pig. Hart, Steenbock and Ellis ('20) have reported that 50 ml. of summer milk or 75 ml. of winter milk were required to furnish a minimum protective dose of vitamin C, while Dutcher, Eckles, Dahle, Mead and Schaefer ('20) reported that the quantity of milk required to furnish this amount of vitamin C depends upon the diet of the cow and was found to range from 20 to 60 ml.

Results of investigations other than those reported above emphasized the importance of the diet of the cow on the vitamin C content of the milk produced, but a review of the available literature failed to indicate that any systematic

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study had been made concerning the effect of breed characteristics or of stage of lactation on the vitamin C content of milks produced under comparable conditions.

Since milk constitutes an important item in the human diet and since the importance of an adequate amount of vitamin C in the diet is becoming more fully appreciated, it appeared to us that any investigation that might yield further information as to how milks containing greater quantities of this dietary essential might be produced would be worth while.

The more recent investigations concerning the nature and the reactions of vitamin C (ascorbic acid) (King and Waugh, '32; Waugh and King, '32; Harris and Ray, '33; Herbert, Hirst, Percival, Reynolds and Smith, '33; Reichstein, Grussner and Oppenauer, '33; Ault, Baird, Carrington, Haworth, Herbert, Hirst, Percival, Smith and Stacey '33; Bessey and King, '33) appeared to suggest a more direct technic that might be used in carrying out such an investigation. This, together with the fact that the college herd is composed of cows of the various standard breeds, representing different stages of lactation and receiving a uniform diet of definite composition, offered further inducement toward such a study. In fact many of the essentials for a comprehensive study of the effects of both breed characteristics and stages of lactation on the vitamin C content of cow's milk were available.

EXPERIMENTAL

Samples of freshly drawn milk (morning) from cows comprising the college herd were collected and taken directly to the laboratory where the determination of their ascorbic acid content was started at once. These cows were receiving a typical dairy ration which contained weighed quantities of green feed. The ascorbic acid content of the various samples of milk was determined in the following manner: 25 ml. of the fresh milk were treated with 25 ml. of a 16 per cent trichloracetic acid solution in order to precipitate the proteins. The supernatant liquid was removed from the precipitated materials by centrifuging and decanting. The precipitated residue was then washed successively with 20 and 10 ml.

portions of an 8 per cent trichloracetic acid solution. These washings were combined with the supernatant liquid and the total volume titrated with a standardized solution of 2–6 dichlorophenolindophenol. This 2–6 dichlorophenolindophenol was prepared by dissolving 0.1 gm. of the dye in successive portions of warm distilled water, filtering and diluting to 200 ml. The filtered dye solution was then standardized against a N/100 iodine solution and also against pure ascorbic acid before being used. A fresh dye solution was prepared for each day's titration.

DATA

Since space does not permit the presentation of data obtained from each individual cow used in the studies pertaining to the effects of breed characteristics on the ascorbic acid content of the milk, only the data from cows selected as representative of each of the five respective breeds are presented in table 1. In selecting these representative cows, due consideration was given to such factors as stage of lactation, age of cow, milk production, etc. In addition, all cows which showed any detectible evidence of having mastitis were eliminated from consideration. When considered, therefore, from the standpoint of these factors, the cows comprising the several groups were as nearly representative of the respective breeds as was possible to select from the number comprising the college herd.

In the studies relative to the effect of the stage of lactation on the ascorbic acid content of milk, data from all cows were considered in the tabulation. That, is, all cows regardless of breed, age, milk production, etc., were tabulated in monthly groups in accordance with the stages of lactation. The average ascorbic acid contents of the milks for the various stages of lactation were then calculated in terms of milligrams per quart and are presented graphically in figure 1.

DISCUSSION

In the studies relative to the influence of breed characteristics on the ascorbic acid content of cow's milk, the milks

TABLE 1

Giving the ascorbic acid content of a series of milk samples produced by representative cows from five dairy breeds

BREED	NUMBER OF COW	APPROXIMATE AGE OF COW AT START OF EXPERIMENT	ASCORBIC ACID CONTENT OF MILK PER QUART						
			Sample taken 8/1/34	Sample taken 8/4/34	Sample taken 8/11/34	Sample taken 8/20/34	Sample taken 9/1/34	Sample taken 9/8/34	Average
		months	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Ayrshire	1392	92	10.4	12.1	10.5	11.1	12.6	12.2	11.5
Ayrshire	1439	81	14.7	14.6	13.9	17.0	14.2	15.3	14.9
Ayrshire	1477	77	14.3	10.6	12.2	12.5	12.7	12.0	12.4
Ayrshire	1754	39	14.9	12.6	10.6	14.8	14.7	12.2	13.3
Ayrshire	1758	39	8.4	10.0	10.8	13.3	13.3	12.2	11.3
Ayrshire	1770	37	15.9	12.9	13.9	14.2	13.9	12.5	13.9
Average for the breed		61	13.1	12.1	12.0	13.8	13.6	12.7	12.9
Brown Swiss	1551	72	14.6	15.2	17.7	15.2	15.3	12.2	15.0
Brown Swiss	1559	70	11.6	11.6	9.7	10.5	16.3	13.8	12.2
Brown Swiss	1611	64	13.3	14.8	12.2	12.5	13.5	13.4	13.3
Brown Swiss	1615	64	17.6	19.8	19.2	22.0	20.0	18.3	19.5
Brown Swiss	1619	67	12.5	11.5	10.5	12.0	14.6	12.0	12.2
Brown Swiss	1734	42	15.9	15.0	15.7	16.9	16.8	18.0	16.4
Average for the breed		63	14.3	14.6	14.2	14.9	16.1	14.6	14.8
Guernsey	1484	118	12.7	12.6	14.6	16.0	17.1	14.2	14.5
Guernsey	1545	79	12.6	11.5	11.1	13.1	14.7	12.4	12.6
Guernsey	1548	76	11.9	10.5	11.8	13.1	13.5	11.7	12.1
Guernsey	1767	40	14.6	14.9	11.2	11.4	12.5	12.4	12.8
Guernsey	1771	37	18.5	14.3	14.3	16.5	16.3	17.1	16.2
Guernsey	1797	34	12.5	11.4	8.9	7.0	9.9	8.4	9.7
Average for the breed		64	13.8	12.5	12.0	12.8	14.0	12.7	13.0
Holstein	1440	83	9.4	10.7	9.4	9.8	9.8	10.0	9.9
Holstein	1646	58	9.8	10.1	10.0	11.2	11.3	11.7	10.7
Holstein	1656	56	9.9	8.3	10.1	8.5	10.3	10.0	9.5
Holstein	1698	52	11.7	9.5	8.6	8.6	11.3	12.7	10.4
Holstein	1744	41	11.0	10.2	11.3	12.5	10.5	10.1	10.9
Holstein	1805	32	9.2	7.0	7.3	10.8	8.4	7.8	8.4
Average for the breed		54	10.2	9.3	9.4	10.2	10.3	10.4	10.0
Jersey	1480	74	15.2	15.4	14.2	15.3	15.2	15.9	15.2
Jersey	1501	77	10.1	11.4	10.4	12.9	12.8	10.1	11.3
Jersey	1703	112	15.4	14.2	11.3	14.6	13.5	13.9	13.8
Jersey	1717	69	15.8	14.6	14.6	15.1	17.1	19.3	16.1
Jersey	1793	53	13.1	12.5	10.0	8.4	14.1	12.1	11.7
Jersey	1821	46	12.1	13.5	12.4	7.4	12.1	11.8	11.6
Average for the breed		72	13.6	13.6	12.2	12.3	14.1	13.8	13.3

from a total of seventy-nine cows were collected. This group of cows was composed of: 14 Ayrshires, 13 Brown Swiss, 9 Guernseys, 27 Holsteins and 16 Jerseys. A few of these cows were known to have mastitis, but when it was found that mastitic milks gave abnormal ascorbic acid values such cows were eliminated from consideration. Milks from sixty-one cows were titrated at each of the six titration periods, while milks from eleven other cows were titrated from two to five

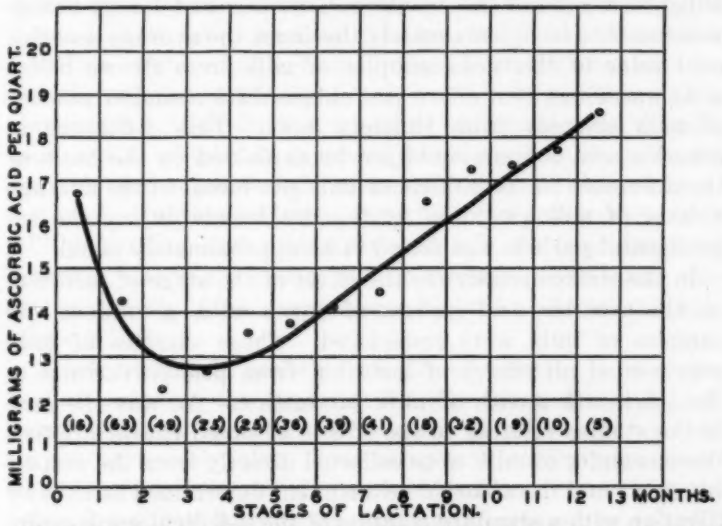

Fig. 1 Showing the effect of the stage of lactation on the ascorbic acid content of the milk produced. The numbers given in enclosure represent the number of samples of milk titrated during the respective periods.

times during the 39-day experimental period. These cows ranged from 3 to 10 years of age and represented all stages of lactation.

It may be observed from the data given in table 1 that the ascorbic acid content of the various milk samples varied with the cow almost as much as it varied with the breed. It may also be observed from the data given in this table that the ascorbic acid content of milk from some cows showed slight variations from period to period. While no explanation is

offered for the latter variation, it seems highly probable that at least part of the variation in the ascorbic acid content of milks produced by cows of the same breed is due to different stages of lactation.

The data presented, while not showing sharp differences in the ascorbic acid content of milks from cows of different breeds, indicate that slight differences do exist in the case of certain breeds. While the average ascorbic acid content of milks from cows of the Ayrshire, Guernsey and Jersey breeds was found to be approximately the same, the average ascorbic acid value of thirty-six samples of milk from Brown Swiss cows was 48 per cent above that obtained for a similar number of milk samples from Holstein cows. This difference in ascorbic acid content could not be explained on the basis of the difference in the volume of milk produced, as the average volume of milk produced by the two breeds during the experimental periods was found to be approximately equal.

In the studies relative to the effect of the stage of lactation on the ascorbic acid content of cow's milk, a total of 410 samples of milk were considered. These samples of milk represented all stages of lactation from post-parturition to the thirteenth month of milk production. As was the case in the studies relating to the effects of breed characteristics, these samples of milk were collected directly from the respective cows and the ascorbic acid content determined at once by titration with a standard solution of the 2–6 dichlorophenolindophenol. In order to conserve space, the data obtained in this phase of the investigation have been arranged in accordance with the stage of lactation of the respective cows and are presented in graphic form.

It will be observed from these data (fig. 1) that the average ascorbic acid content of milks produced by a mixed dairy herd, receiving similar rations, varied widely according to stages of lactation. Eighteen samples of such milk, produced during the first month of lactation, were found to contain an average of 16.7 mg. of ascorbic acid per quart, while forty-nine samples of milk produced during the third month of lactation contained an average of only 12.3 mg. per quart, and

five samples of milk produced during the thirteenth month of lactation contained 18.6 mg. per quart. Samples of milk taken at intermediate stages of lactation gave intermediate ascorbic acid values.

A few samples of colostrum were also titrated and in each case the ascorbic acid content was found to be unusually high. This high ascorbic acid content of colostrum was believed to indicate a certain degree of storage of this substance by the cow during the pre-parturition period. The data presented in figure 1 show, however, that if such a body-store of ascorbic acid does exist it must be limited, since 2 months after parturition the amount of this substance in the milk has dropped to a minimum. After the first 2 months of lactation, the ascorbic acid content of milk is apparently dependent solely upon the ascorbic acid content of the diet of the cow. With a constant intake of ascorbic acid in the diet of the cow and a decrease in milk production such as usually occurs in the later stages of lactation, one might expect milk of higher ascorbic acid content, especially if the storage capacity of the cow for this substance is limited.

A point which is probably worthy of mention in this connection concerns the apparent relation between 'cardboard-flavor' and low ascorbic acid content of milks. It was observed, invariably, that those samples of milk, portions of which had been titrated for ascorbic acid content, which developed cardboard-flavor on standing, decreased in ascorbic acid content coincident with the formation of the off-flavor. Whether cardboard-flavor is due to the degradation products of ascorbic acid or whether those factors instrumental in the formation of cardboard-flavor are also instrumental in the destruction of ascorbic acid was not determined.

SUMMARY

A study has been made concerning the effects of breed characteristics and stages of lactation on the ascorbic acid content of cow's milk. As a result of these studies, the following conclusions are drawn:

1. Cows of the same breed, while receiving similar diets, may produce milks which show wide variations in their ascorbic acid content. Such variations in ascorbic acid content are thought to be due, in part at least, to differences in stages of lactation.

2. Cows of different breeds, while receiving similar diets, produced milks which differed somewhat in their average ascorbic acid values. Of the five breeds studied, the Brown Swiss cows produced milks of the highest ascorbic acid content, while the Holstein cows produced milks of the lowest ascorbic acid value.

3. Stage of lactation appeared to have a more definite effect upon the ascorbic acid content of milk than did breed differences. The ascorbic acid content of milk was found to be relatively high during the early stages of lactation, but decreased to a minimum after about 2 months of lactation, and then increased to a maximum in the later stages of lactation.

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THE EFFECT OF CELLULOSE, HEMICELLULOSE AND LIGNIN ON THE WEIGHT OF THE STOOL: A CONTRIBUTION TO THE STUDY OF LAXATION IN MAN

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TWO FIGURES

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Of late we have been concerned with the physiological effects of the indigestible residue fraction of foods (lignin, cellulose and hemicellulose). In a previous paper (Williams and Olmsted, '35) we demonstrated by a new method that 'crude fiber' is not a chemical entity. Our method, which determines separately lignin, cellulose and hemicellulose, made possible a reinvestigation of: First, the determination of these products in foods containing indigestible residues; second, the fate of each of these fractions when fed to human subjects, and third, the effect of cellulose, lignin and hemicellulose on the bulk of the stool.

As a corollary to our experiment the problem of the utilization of indigestible residues should be attacked. For at least 70 years attempts have been made to determine how much cellulose is broken down in the gastro-intestinal tract of mammals. The problem is of special interest in animal husbandry in that the economy and nutrition of ruminant herbivores is involved. It is estimated (McCance and Lawrence, '29) that a considerable part of the caloric intake of a cow comes from bacterial ferment digestion of residues

in the rumen. The suggestion has been made that man obtains nutriment from intake of indigestible residue in an analogous manner. McCance and Lawrence concluded, however, that from this standpoint the indigestible carbohydrate portion in the human diet was of little importance. The progress of the problems in reference to the utilization of crude fiber has been excellently reviewed by Mangold ('34). For the most part, workers have been unable to surmount the unsuitability of analytical methods. A serious objection to previous work is in the pre-treatment of the materials fed. It is obvious that vigorous chemical treatment, as in preparing Weende crude fiber, profoundly alters its composition and the resultant digestibility data are meaningless when applied to naturally occurring residues. In the light of the foregoing criticism we doubted the validity of the results obtained in one of our studies (Olmsted, Curtis and Timm, '35) of human subjects who were fed pentosan extracted from wheat bran by means of strong alkali. In the present study this doubt is confirmed; our results indicate that a chemical pre-treatment profoundly alters the extent to which indigestible residue disappears from the gut.

So far, it is generally agreed that the end products of carbohydrate residue disintegration in the digestive tract are soluble sugars, fatty acids, gases (methane, hydrogen, carbon dioxide) and alcohols. Symbiotic bacterial enzymes evidently hydrolyze cellulose and hemicellulose into soluble products which either are absorbed as such or are immediately fermented to gases and fatty acids of the lower hydrocarbon series. It seems that the initial reaction is the determinant, for only by means such as Pringsheim ('23) used can intermediary products be isolated. Pringsheim stopped the reaction in an intermediary stage and detected cellobiose (an intermediary between cellulose and reducing sugars-maltose and dextrose) and reducing sugars. Khouvine ('23) isolated from the human gastro-intestinal tract a cellulose splitting bacterium in pure culture. In vivo the case is not as simple. Certainly in this experiment we found a very

small quantity of copper reducing, sugar-like substances and an abundance of volatile fatty acids. For this reason we quantitatively determined volatile fatty acids which are easily determinable end products of indigestible residue breakdown.

Bahrdt and Edelstein ('11) showed that lower volatile fatty acids when fed to dogs increase the volume of the stool. Grove, Olmsted and Koenig ('29) indicated that high carbohydrate diets augment the stool weight more than either high fat or high protein diets. Pediatricians have recognized the efficacy of added sugar in infant feeding to promote laxation. Grove, Olmsted and Koenig further showed that by high carbohydrate diets the stool volatile fatty acids could be tremendously increased; but they also demonstrated that stool volatile fatty acids can be increased in human subjects by simple mineral catharsis. Therefore, it appears that a high volatile fatty acid content in the stool either accompanies an increased stool volume or is the cause of it. There is considerable evidence from our experiment that the relationship is not merely a concomitant one but definitely causal in nature.

EXPERIMENTAL

In outline, our plan was to isolate indigestible residues from naturally occurring sources with as little change as possible in their original compositions (subsequent analysis showed no essential change). We fed these prepared materials to human subjects on a fixed basal diet free from indigestible residue; analyzed the resultant stools for indigestible residue, volatile fatty acids and soluble hydrolyzable reducing substances and noted the subjective effects. We recorded the wet weight of the stool as passed, and from this value subtracted the average (of three) basal period stool weights plus the weight of the residue which had been recovered in the feces. This value we define as the *increment of stool weight.*

1. Preparation of feeding materials

Our object in pre-treatment of the materials was to concentrate the indigestible residue by removal of water, starch, sugars, minerals, oils, fats and resins. By concentration we obtained more nearly homogenous samples in manageable quantities and eliminated substances which might complicate the experiment.

We obtained from the open market carrots, cabbage and canned peas. After grinding in a food chopper to about 20 mesh (dry) each material was suspended in a mechanical washer and washed for 24 hours at 65°C. The washer was of this type: The material was inclosed in a 20-mesh wire gauze cylinder which in turn was suspended in a slightly larger cylinder of sheet metal. The water was forced spirally from the bottom of the inner cylinder and at such a rate that the material was thoroughly mixed and suspended during the washing time.

Wheat, bran, alfalfa leaf meal, corn germ meal, cotton seed hull meal and sugar beet pulp were secured from the mill[1] already ground. These were washed as were the vegetables.

Subsequently each of the materials was quickly air dried at about 40°C. and then extracted with boiling ethyl alcohol in a Soxlet apparatus for 24 hours. The alcohol was evaporated and the materials again air dried. In addition to the materials listed above, cellu flour[2] (40 mesh) and agar agar (20 mesh) were fed without additional preparation, for analysis showed that additional treatment was unnecessary.

Analyses of the materials before and after pre-treatment indicated that the process did not alter the essential composition of the indigestible residue but did remove most of the water, starch, soluble hemicelluloses, sugars, minerals, fats, oils, resins and coloring matter, and thus concentrated the residue. The pre-treatment was not made quantitative but 60 pounds of carrots, 60 pounds of cabbage and 42 cans (no. 2) peas were used to obtain the indigestible residue fed during the vegetable periods.

[1] Courtesy of the Ralston Purina Co., St. Louis, Mo.

[2] A product of the Chicago Dietetic Supply House.

We attempted the preparation of linseed meal and canned corn but found that these necessitated an enzymatic treatment in addition to the process described above. Since their residue composition so closely resembled that of other materials on our list, we did not feed these two products.

2. Selection of subjects

We selected three male medical students whose colons by past history and x-ray studies appeared normal but differed in type. Subject 'W' was hyposthenic, subject 'F' was hypersthenic and subject 'H' was intermediary. This selection was made in view of the theory that colonic type influences the coefficient of utilization of indigestible residue and the stool volume.

3. Basal diets

All meals were prepared in the metabolism ward kitchen of Barnes Hospital. The foods used were saccharose, strained orange, lemon and grapefruit juices, meats, dairy products, white flour in bread, spaghetti, noodles and jello. *No vegetables or materials containing indigestible residue were fed.* We did not feed charred meats or toast and the crust of bread was removed. This precaution is necessary, for carbon apppears quantitatively in the lignin fraction of stools. Outside the basal diet we allowed only water, ginger ale and small quantities of black coffee (unsweetened). Subsequent analyses of the stools from the basal diet indicated an inconsiderable amount of indigestible residue. (Per week, subject 'F' 1.8 gm.; subject 'W' 1.6 gm.; subject 'H' 2.9 gm.) In our analysis the residue was fractionated into lignin, cellulose and hemicellulose. Although the basal residue represented a very small fraction of the material recovered from a feeding period, it was subtracted from the total recovery. That the basal diet was adequate calorically is indicated by the fact that the subjects' weights were maintained.

4. Method of feeding

The basal diet was fed for 7 days (Monday through Sunday). The total amount of each prepared material was distributed over 6 days (Monday through Saturday); and the stools were collected for 7 days (Tuesday through Monday). The basal diet was repeated precisely to the day and to the meal during each of the feeding periods, and was repeated alone on the sixth and tenth weeks: viz., basal, four feeding periods; basal, three feeding periods; basal, three feeding periods—a total of 13 weeks. As a rule the material was fed as a dry cereal with cream and sugar.

The amount of material fed was determined in this way: In the past the general rule has been to determine the Weende crude fiber value of a material, to feed it irrespective of its chemical composition and to determine, by the same procedure, the crude fiber in the stool. In our previous paper ('35) we indicated the inadequacies of this method, for Weende crude fiber represents a variable fraction of the total indigestible residue; and what becomes of the fraction of residue not determined as crude fiber is not known. Furthermore, it is in no wise true that material which has passed through the gut is as susceptible to chemicals as the original material. We believe that it is more susceptible and that the loss attributed to utilization may have been partially apparent. Certainly we have found that consecutive Weende treatment of crude fiber causes additional losses in the lignin and hemicellulose fractions. Our method of analysis makes possible the fractionation of indigestible residue and the separate following of each component in the gut.

Since we did not know the relative influence of the three components (lignin, cellulose and hemicellulose) on the volume of the stool, but suspected that lignin and cellulose were the important fractions, we kept these two constant and fed 10 gm. of lignin plus cellulose per day with the hemicellulose as the variable. Our results did not bear out this hypothesis, for hemicellulose proved very important not only in its break-

down but also in the hydroscopic properties of residues which we believe to be attributable chiefly to the hemicellulose fraction.

Table 1 presents the analyses of materials quite different in their composition of indigestible residue. The cellulose ranges from 0 per cent to 78 per cent, lignin from 0 per cent to 21 per cent and hemicellulose from 9 per cent to 81 per cent. In table 1 hemicellulose is divided into two fractions. This

TABLE 1

Analysis of materials fed

MATERIALS ADDED TO THE BASAL DIET	WHEAT BRAN	ALFALFA LEAF MEAL	CARROTS	CORN GERM MEAL	COTTON SEED HULL	SUGAR BEET PULP	CANNED PEAS	CABBAGE	AGAR AGAR	CELLU FLOUR
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Cellulose	16.9	32.5	23.2	15.8	19.4	34.2	35.0	29.5	0.0	78.8
Lignin	7.8	15.0	3.4	2.4	20.8	2.5	1.7	2.6	0.0	0.0
Hemicellulose (S)	35.2	15.5	20.7	28.9	30.9	20.4	8.8	19.8	81.0	16.9
Hemicellulose (L)	0.0	3.7	8.1	1.9	0.6	8.8	2.1	8.5	....	0.0
Starch	6.6	2.5	0.7	10.9	3.2	1.8	13.6	0.0	0.0	0.0
Protein (N×6.25)	12.3	12.4	7.8	18.6	7.3	8.5	16.5	10.8	1.6	0.0
Moisture (110°)	8.9	6.5	11.1	7.5	8.2	7.9	7.5	10.0	13.5	3.0
Ash (red heat)	4.4	2.3	5.8	5.5	1.8	2.3	2.2	3.8	3.4	0.1
Soluble in alcohol-benzene	3.5	9.0	15.2	7.2	7.0	9.8	9.7	12.5	0.0	0.0
Total	95.6	99.4	96.0	98.7	99.2	96.2	97.1	97.5	99.5	98.8

(L) Soluble in solutions pH 8.
(S) Insoluble in solutions pH 8.

division is necessitated by the fact that the term hemicellulose merely designates a group, some of whose members are resistant to concentrated alkali and others are susceptible to the dilute alkali (pH 8) which is used in our method of analysis. This latter fraction is variable but usually quite small, and is called the labile fraction (L). Bran and alfalfa leaf meal have the same ratio of cellulose to lignin; but for 10 gm. of lignin plus cellulose, bran has four times as much hemicellulose (table 2). Carrots and corn germ meal have the

same cellulose to lignin ratio; but for 10 gm. of cellulose plus lignin, corn germ meal has twice as much hemicellulose. From these two pairs one should be able to determine the influence of lignin on the breakdown of cellulose and hemicellulose and the influence of hemicellulose on stool volume. Cotton seed hull meal is an example of a material with an exceedingly low ratio of cellulose to lignin; and if lignin influences the breakdown of cellulose and hemicellulose, this material should indicate the fact. Agar agar is an example of a material containing only hemicellulose. Cellu flour is an

TABLE 2

Amounts of materials fed to each subject during each 6-day period

MATERIALS ADDED TO THE BASAL DIET	MATERIAL FED	LIGNIN	CELLULOSE	HEMICELLULOSE	TOTAL INDIGESTIBLE RESIDUE
	gm.	*gm.*	*gm.*	*gm.*	*gm.*
Wheat bran	243.0	18.9	41.1	86.0	146.0
Alfalfa leaf meal	126.5	19.0	40.9	19.6	79.5
Carrots	226.1	7.7	52.3	46.8	106.8
Corn germ meal	328.9	7.8	51.9	95.0	154.8
Cotton seed meal	149.0	31.0	28.9	46.0	105.9
Sugar beet pulp	163.5	4.1	55.9	33.4	93.4
Canned peas	163.8	2.7	57.3	14.4	74.5
Cabbage	210.6	5.5	54.5	41.5	101.5
Agar agar	99.0	0.0	0.0	80.2	80.2
Cellu flour	84.0	0.0	60.5	11.2	71.7

example of a material prepared analogously to Weende crude fiber or filter paper, materials which have long been fed to increase the stool weight. Table 2 gives the exact amounts of materials fed. Although the total amounts of materials fed varied from 84 to 329 gm., for 6-day periods, the amounts of cellulose plus lignin were in all instances 60 gm.

5. Collection of stools

Each stool was collected free from urine, weighed as passed; the wet weight was multiplied by 5 and this number of cubic centimeters of distilled water added. The stool was then thoroughly mixed by a mechanical mixer, put in a fruit

jar and steam sterilized (15 pounds steam pressure, 30 minutes), sampled aseptically and the sample analyzed immediately or stored under toluol at 6 to 10°C. Since the feeding was discontinued Saturday at 9 A.M. and the last stool collected at 9 A.M. Monday, analysis of the stools showed that essentially 100 per cent of the material to pass the gut came through in that time (48 hours). We did not use charcoal or carmine to mark the stools; but during the first five periods we analyzed the stools on the third, sixth and seventh days of stool collection. Since the results of these periods showed nothing to be gained by so many separate analyses, we pooled the 7-day stools of each subject and made one analysis in triplicate of each period.

6. *Methods of analyses*

Indigestible residue in the materials fed[3] and in the stools was determined by the method of Williams and Olmsted ('35) with the exception that, since the materials were already graded to 20 mesh, no additional comminution was neceessary.

Volatile fatty acids were determined by the method of Olmsted et al. ('29) on stool filtrates prepared by the procedure of Steiner, Urban and West ('32) using ferric sulphate and Lloyd's reagent as the precipitating agent. We used 60 cc. stool suspension (1:6) and 25 cc. ferric sulphate with a final dilution of 250.

Soluble sugar or sugar-like substances were determined on the West filtrate both before and after hydrolysis (1.5 N H_2SO_4; 2 hours; 100°C.) by the technic of Shaffer and Somogyi ('35) and the fermentation procedure of Somogyi ('28). Exploratory analyses indicated that the copper re-

[3] Agar agar cannot be analyzed by our usual procedure. Instead of the enzymatic pre-treatment followed by strong acid hydrolysis we hydrolyzed it by 2.5 N. H_2SO_4, 100°C., 3 hours. Subsequent determination of reducing sugars follows our original method. Agar stools were thus hydrolyzed and then precipitated by the West method (Steiner, Urban and West, '32) before analysis of the reducing sugars in the hydrolysate. Basal period stools were treated identically and their very small residue values subtracted from the agar period residues.

ducing value of the West ferric sulphate reagent filtrate was lower than that of either the mercuric sulphate (West, Scharles and Peterson, '29) or the mercuric-ferric sulphate (E. S. West, unpublished) reagent filtrates and that the volatile fatty acids were identical in all three. We concluded, therefore, that the high acid concentration of the mercuric sulphate reagent hydrolyzed some of the more labile residue. With the ferric sulphate reagent combined with Lloyd's reagent filtrate we found only traces of reducing substances either before or after acid hydrolysis of the filtrate.

Criteria of increased stool volume: We agree with Cowgill and Anderson ('32) that the impression of the subject is an important criterion; and we attempted to rate our materials accordingly. Most certainly the volume of the stool, its dry weight or its wet weight are not the only criteria. Our subjects reported considerable difficulty in passing stools from such materials as alfalfa leaf meal, cotton seed hull meal, cellu flour, and to a certain extent, wheat bran. Here the stool weight and stool volume were quite high but the feces were dry, hard and often flecked with blood. An objective rating, which coincides remarkably well with the subjective rating, is what we term the increment in stool weight which we have already defined.

Inasmuch as the results of this study revealed a direct and unexpected relation between the increment in the stool weight and the *percentage disappearance* of ingested food residue from the gut instead of *percentage recovery* in the stool, the data presented in tables 3 and 4 and figure 2 pertain to disappearances rather than recoveries.

RESULTS

The results are given in tables 3, 4, 5 and 6.

Table 3 gives the percentage disappearance of each of the three fractions of indigestible residue (lignin, cellulose and hemicellulose) for each of the three subjects. These observations can be made: 1) The percentage disappearance of any one material is strikingly uniform for the three subjects.

2) In general, the magnitude of the percentage disappearance is in this order: Hemicellulose, cellulose and lignin. The conclusion is based on only those materials containing large percentages of lignin. 3) The percentage of lignin in naturally occurring materials profoundly influences the percentage disappearance in the human gut. This is most striking when cotton seed hull meal (high in lignin) is compared with other substances lower in lignin; or when the group high

TABLE 3

Percentage disappearance of materials fed

MATERIALS ADDED TO THE BASAL DIET	SUBJECT F				SUBJECT W				SUBJECT H			
	Lignin	Cellulose	Hemicellulose	Total indigestible residue	Lignin	Cellulose	Hemicellulose	Total indigestible residue	Lignin	Cellulose	Hemicellulose	Total indigestible residue
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Wheat bran	10.5	31.6	33.6	30.0	4.0	24.0	32.0	26.2	16.0	33.0	39.0	34.2
Alfalfa leaf meal	5.5	11.2	9.0	9.5	0.0	12.5	0.0	7.0	4.0	13.0	7.6	10.0
Carrots	55.8	72.5	88.9	79.4	50.6	62.3	80.0	69.2	54.6	65.6	84.6	73.1
Corn germ meal	44.9	55.4	62.5	59.3	55.0	62.0	67.4	64.9	41.0	53.4	57.9	55.6
Cotton seed hull meal	23.5	23.9	37.4	29.6	3.0	3.2	14.2	7.2	10.0	25.0	39.5	17.5
Sugar beet pulp	36.5	41.8	88.3	55.0	17.0	62.8	90.4	70.7	36.6	61.4	88.0	69.8
Canned peas	63.6	40.0	79.9	48.4	66.6	44.8	86.2	53.4	39.3	49.0	86.8	55.8
Cabbage	72.7	61.3	79.0	69.2	54.5	43.5	80.5	59.3	69.1	59.2	80.2	68.4
Agar agar	....	....	65.5	65.5	....	....	60.2	60.2	....	....	55.2	55.2
Cellu flour	....	10.9	47.7	15.9	....	10.0	28.0	13.0	....	0.0	10.8	2.0

in lignin (cotton seed hull, wheat bran, alfalfa leaf) is compared with the group low in lignin (carrots, corn germ, peas, beet pulp, cabbage). This confirms the original postulate of Waentig and Gierisch ('18). 4) The slight disappearance of cellu flour would seem to indicate that processing remarkably lowers the percentage disappearance. If energy values and utilization are considered, it would seem to be indicated that our human subjects derived little nutritive value from the indigestible residue fed. We fed very nearly the maximum amount of indigestible residue, for with certain materials

there was at least one exceedingly large stool per day. Larger amounts would have caused diarrhea. With corn germ meal 89 gm. of cellulose plus hemicellulose disappeared in 6 days. This represents 14.5 gm. per day from which could be obtained only a very small fraction of the 2300 to 2800 Cal. daily needs of our subjects.

Since it has been suggested that volatile fatty acids may be a stimulus to the gut, we attempted to correlate the increment (over basal) in stool volatile fatty acids with the increment in stool weight and the cellulose plus hemicellulose disappearing. Table 4 presents in detail the data of the three subjects. Attention is again called to the close agreement between the three subjects.

Table 5, a composite of the data from the three subjects, relates the increment in stool weight, the amount of indigestible residue fed and the residue remaining in the stools. Figure 1 graphically presents the data of table 5.

These observations can be made: 1) The increment in stool weight does not correspond to the amount of residue fed. 2) An inverse relationship exists between the increment in stool weight and the material recovered in the stool, i.e., the less material recovered the greater the increment in the stool weight. It would appear, therefore, that the amount disappearing from the gut determines the true increase in stool volume.

It may be objected that the increment in stool weight represents merely water absorbed by the residue not disappearing from the gut, i.e., the hydroscopic properties of the residue. However, column 5 of table 5 would answer this objection in that, for instance, each gram of agar agar would have increased the stool weight 20 gm., each gram of cabbage 18 gm., each gram of carrots 19 gm., and each gram of sugar beet pulp 12 gm. To us this hypothesis seems untenable and we conclude that some factor in addition to the hydroscopic properties of the stool residue must account for the increment in stool weight.

TABLE 4

Increment in stool weight related to increment in volatile fatty acids and cellulose plus hemicellulose disappearing

MATERIAL ADDED TO BASAL DIET	SUBJECT F					SUBJECT W					SUBJECT H				
	Increment stool volatile fatty acids (1.0 N alkali)	Increment stool weight	Cellulose plus hemicellulose disappearing			Increment stool volatile fatty acids (1.0 N alkali)	Increment stool weight	Cellulose plus hemicellulose disappearing			Increment stool volatile fatty acids (1.0 N alkali)	Increment stool weight	Cellulose plus hemicellulose disappearing		
			Hemicellulose	Cellulose	Total			Hemicellulose	Cellulose	Total			Hemicellulose	Cellulose	Total
	cc.	*gm.*	*gm.*	*gm.*	*gm.*	*cc.*	*gm.*	*gm.*	*gm.*	*gm.*	*cc.*	*gm.*	*gm.*	*gm.*	*gm.*
Wheat bran	89.4	575	28.9	13.1	41.0	98.7	578	27.2	9.9	37.1	116.0	373	33.5	13.4	46.9
Alfalfa leaf meal	12.4	182	1.8	4.6	6.4	36.7	318	0.0	5.2	5.2	34.5	221	1.5	5.4	6.9
Carrots	104.4	700	41.6	37.9	79.5	109.7	589	37.4	32.6	70.0	77.0	336	39.6	34.3	73.9
Corn germ meal	98.4	590	59.4	28.8	88.2	84.2	563	64.0	32.2	96.2	148.0	581	55.1	27.7	82.8
Cotton seed hull meal	7.9	104	17.2	6.9	24.1	52.7	123	6.6	0.2	6.8	24.0	66	8.2	7.3	15.5
Sugar beet pulp	178.4	662	29.5	23.4	52.9	75.7	372	30.2	35.2	65.4	40.0	171	29.4	34.3	63.7
Canned peas	60.9	114	11.5	22.8	34.3	34.7	255	12.4	25.5	37.9	38.8	134	12.5	28.1	40.6
Cabbage	136.4	647	32.8	33.4	66.2	158.7	781	33.4	23.7	57.1	110.5	449	33.3	32.3	65.6
Agar agar	60.4	835	53.4	....	53.4	98.0	719	48.3	....	48.3	110.5	445	44.3	....	44.3
											(—)	(—)			
Cellu flour	3.4	223	5.3	6.3	11.6	9.7	119	3.1	6.2	9.3	2.5	63	1.2	0.2	1.4

TABLE 5

Relationship of increment in stool weight to residue fed and residue remaining in the stool

MATERIALS ADDED TO THE BASAL DIET	INDIGESTIBLE RESIDUE FED (1)	INDIGESTIBLE RESIDUE REMAINING IN STOOL (2)	INCREMENT IN STOOL WEIGHT (3)	RATIO INCREMENT STOOL WEIGHT TO WEIGHT OF RESIDUE FED (3)/(1) (4)	RATIO INCREMENT STOOL WEIGHT TO INDIGESTIBLE RESIDUE IN STOOL (3)/(2) (5)
	gm.	*gm.*	*gm.*		
Wheat bran	146.0	102.0	475	3.25	4.65
Alfalfa leaf meal	79.5	72.5	240	3.04	3.00
Carrots	106.8	27.8	541	5.62	19.30
Corn germ meal	154.8	62.0	578	4.58	9.34
Cotton seed hulls	105.9	86.6	97	2.06	1.13
Sugar beet pulp	93.4	32.5	401	4.92	12.30
Canned peas	74.5	35.3	167	3.27	4.77
Cabbage	101.5	34.9	625	6.91	17.90
Agar agar	80.2	32.9	666	8.75	20.80
Cellu flour	71.7	64.2	93	2.34	1.45

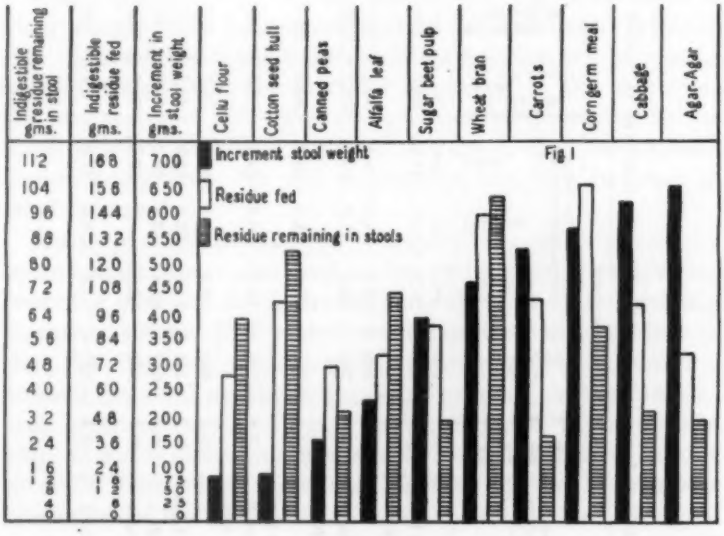

Figure 1

Table 6, a composite from the data of table 4, relates the increment in stool weight, the cellulose plus hemicellulose disappearing and the increment in stool volatile fatty acids. The subjective estimation of the relative values of the materials is also correlated. Figure 2 presents graphically the relationships.

It will be observed that the values of the residue breakdown and the increment in stool volatile fatty acids roughly parallel the increment in stool weight. This then would seem to indicate that breakdown of residue is the important factor; and, since the increment in volatile fatty acids so closely parallels the disappearance of cellulose and hemicellulose, one might reasonably conclude that the acids come from the breakdown of residue and in turn are responsible for the increment in stool weight. If the residue remaining in the stool is very hydroscopic, for instance agar agar, one might expect the increment in stool weight to be more than commensurate with the breakdown of residue and the increment in volatile acids. This may explain the increment in stool weight in the case of agar agar which appears to be greater than the breakdown

TABLE 6

Increment in stool weight related to hemicellulose plus cellulose disappearing and to increment in stool volatile fatty acids

MATERIALS ADDED TO THE BASAL DIET	INCREMENT IN STOOL WEIGHT	HEMICELLULOSE PLUS CELLULOSE DISAPPEARING			INCREMENT IN STOOL VOLATILE FATTY ACIDS (1.0 N ALKALI)	ESTIMATION BY SUBJECTS OF RELATIVE LAXATION VALUES (1 LEAST; 10 MOST)
		Hemicellulose	Cellulose	Total hemicellulose plus cellulose		
	gm.	*gm.*	*gm.*	*gm.*	*cc.*	
Wheat bran	475	29.8	11.4	41.2	101.3	5
Alfalfa leaf meal	240	1.1	5.0	6.1	27.8	1
Carrots	541	39.5	34.9	74.4	97.0	9
Corn germ meal	578	59.5	29.5	89.0	110.2	8
Cotton seed hull	97	10.6	4.8	15.4	28.2	3
Sugar beet pulp	401	29.7	30.9	60.6	98.0	7
Canned peas	167	12.1	25.4	37.5	44.8	4
Cabbage	625	33.1	29.8	62.9	135.2	10
Agar agar	666	48.6	....	48.6	89.6	6
Cellu flour	93	3.2	4.2	7.4	3.5	2

of residue and the production of volatile fatty acids would lead one to expect.

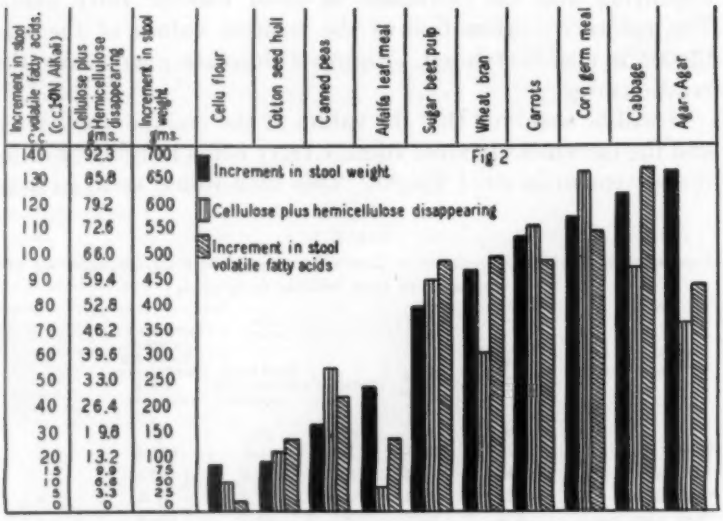

Figure 2

SUMMARY AND CONCLUSIONS

1. The indigestible residues (lignin, cellulose and hemicellulose) found in ten food substances of widely varying sources were concentrated by simple procedures which would not alter the essential composition of the residue. These were added to a non-residue diet and fed to three human subjects.

2. By analysis of the feces it was found that hemicellulose disappeared in larger amounts than cellulose and that lignin disappears least.

3. When there was a high percentage of lignin in the residue, less hemicellulose and cellulose disappeared from the gut.

4. A comparison of the stool weights after feeding of these residues seemed to indicate that the amount of cellulose and hemicellulose disappearing during the passage through the

human gut influenced the volume of the feces more than the amount of residue fed or the amount recovered in the feces.

5. In general, the quantity of stool volatile fatty acids was greatest when a residue disappeared most during its passage through the gastro-intestinal tract.

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THE VITAMIN G COMPLEX

I. THE NON-IDENTITY OF RAT DERMATITIS DUE TO VITAMIN B_6 DEFICIENCY AND THE DERMATITIS OF HUMAN PELLAGRA

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In this study an examination has been made of the position of the newly discovered components of the vitamin G(B_2) complex in relation to the etiology and treatment of pellagra. Ever since Goldberger and Lillie ('26) reported the discovery of a 'pellagra-like' condition in the rat, apparently traceable to the same dietary deficiency as human pellagra, the hypothesis that vitamin G(B_2) and the pellagra-preventive factor are one and the same entity has been widely accepted. With the aid of this hypothesis as a starting point many studies have been made, of which a considerable number lead to conclusions at variance with the idea that human pellagra is caused by a deficiency of vitamin G(B_2). This has led to much confusion, one result of which is that the vitamin G deficiency hypothesis of the causation of pellagra has been rejected by many workers. This hypothesis was nevertheless accepted by the writer because it appeared to be at least as conformable with past observations on pellagra as any of the other hypotheses which have been advanced.

Recently, however, our knowledge of vitamin G has been greatly extended by the work of György, Kuhn and Wagner-Jauregg ('33) who have shown that vitamin G is not a single substance, but a mixture of lactoflavin with a second substance

which György has since called vitamin B_6. György ('34, '35) has examined the nature and distribution of this second substance and has shown that it is the factor whose absence from the diet will induce the pellagra-like dermatitis in the rat. His observations have been substantially confirmed by Chick, Copping and Edgar ('35) and by Harris ('35). This demonstration that vitamin G is made up of two factors, one of which controls the characteristic dermatitis of the rat,[1] appeared to open up the possibility that some of the confusion existing in this field might be cleared away. The first step necessary for a clarification of the position was to determine whether either or both of the newly discovered components of vitamin G possess pellagra-preventive activity. In the following section an account is given of experimental and clinical work directed to that end.

EXPERIMENTAL

1. The curative action of cornmeal in 'rat pellagra.' In a preliminary examination of the effect of white maize meal on the specific dermatitis of vitamin B_6 deficiency in the rat, three rats with severe symptoms were each given 1 gm. daily of white maize meal and all three recovered completely in 3 to 4 weeks. This result is of key importance in determining whether vitamin B_6 plays any part in the etiology of human pellagra and the observation was accordingly repeated and extended as follows. Sixteen rats at the age of 29 days were placed in screen bottom cages and given the vitamin B-free diet of György (vitamin B-free casein, Harris, 18 per cent; rice starch 68 per cent; butterfat 9 per cent; cod liver oil 1 per cent; salt mixture, McCollum's, 4 per cent) ad libitum. For the first 30 days of the experiment no supplement was added to this diet, but from the thirtieth day onward each

[1] Throughout this paper the term rat dermatitis is used to designate the condition termed 'symmetrical pellagra-like dermatitis' by György ('35) or 'florid dermatitis' by Chick, Copping and Edgar ('35). The nomenclature used is that employed by György; namely, vitamin G(B_2) for the sum of the heat stable fractions of the vitamin B complex, and vitamin B_6 for the fraction which controls the rat dermatitis. Lactoflavin is throughout referred to by that name.

rat was given daily 10 γ of crystalline vitamin B(B_1) and was given twice weekly 20 γ of lactoflavin. On this regimen the rats failed to gain weight, developed the specific dermatitis of vitamin B_6 deficiency and appeared listless and in poor condition generally.

Two matched groups of six rats each were then chosen from the sixteen, three in each group having a moderately severe dermatitis, and three in each group having a very severe dermatitis. Each member of the first group was given 1 gm. daily of white maize meal and each member of the second group 1 gm. daily of yellow maize meal in addition to the vitamin B and lactoflavin. The remaining four rats served as negative controls and received no other dietary supplement in addition to the vitamin B and lactoflavin. The results of this experiment are summarized in table 1.

As will be seen from the table the control rats all lost weight and died with very severe dermatitis after an average survival period of 41 days (commencing from the day on which the experimental animals were first dosed). On the other hand those dosed with 1 gm. daily of either white or yellow maize meal all lived through a dosing period of 35 days and gained weight. All rats dosed with yellow maize meal were cured of dermatitis during this period and all except one were in good condition at the close of the experiment. This rat (♂ 00(3)) showed a weight increase above the average and was normally active, but its coat was in bad condition, being extremely thin all over the body. Of the six rats dosed with white maize meal five were cured of their dermatitis and were in good condition at the end of the experiment. The remaining rat (♂ 10(3)) was in poor condition, suffering from an almost complete generalized alopecia, and it still had a slight dermatitis.

From these data it is concluded that 1 gm. of maize meal, whether yellow or white, contains sufficient vitamin B_6 to bring about rapid cure of the characteristic dermatitis of 'rat pellagra' even when it is severe.

TABLE 1

Summary of test of the vitamin B_1 activity of maize meal

A. Rats treated with white and yellow maize meal

RAT	WEIGHT AT 30 DAYS OLD, GRAMS	CONDITION WHEN FIRST DOSED		DOSE	CONDITION AFTER 35 DAYS' DOSING			WEIGHT INCREASE DURING DOSING PERIOD, GRAMS
		Weight, grams	Dermatitis		Weight, grams	Dermatitis	General	
♂ 02(2)	59	46	+++	1 gm. white maize meal	72	0	Good	26
♂ 10(3)	54	44	+++	1 gm. white maize meal	57	+	Poor	13
♂ 01(5)	52	62	++	1 gm. white maize meal	91	0	Good	29
♀ 10(6)	49	49	++	1 gm. white maize meal	71	0	Good	22
♀ 11(6)	46	42	++	1 gm. white maize meal	60	0	Good	18
♀ 02(6)	44	42	+++	1 gm. white maize meal	62	0	Good	20
♀ 00(1)	65	73	+++	1 gm. yellow maize meal	99	0	Good	26
♂ 00(3)	59	49	+++	1 gm. yellow maize meal	77	0	Poor	28
♂ 10(5)	51	62	++	1 gm. yellow maize meal	89	0	Good	27
♂ 11(5)	52	54	++	1 gm. yellow maize meal	80	0	Good	26
♀ 20(6)	44	44	+++	1 gm. yellow maize meal	62	0	Good	18
♀ 21(6)	49	40	++	1 gm. yellow maize meal	54	0	Good	14

B. Control rats

RAT	WEIGHT AT 30 DAYS OLD, GRAMS	CONDITION WHEN EXPERIMENTAL ANIMALS WERE FIRST DOSED		SURVIVAL PERIOD, DAYS	CONDITION AT DEATH		LOSS OF WEIGHT, GRAMS
		Weight, grams	Dermatitis		Weight, grams	Dermatitis	
♂ 20(2)	69	53	+	45	42	+++	11
♀ 10(3)	50	60	++	39	46	+++	14
♂ 21(5)	46	50	++	41	39	+++	11
♀ 20(5)	50	42	+	38	35	+++	7

All rats received the György diet and supplements of pure vitamin B(B_1) and lactoflavin.

+ indicates slight dermatitis.

++ indicates severe dermatitis.

+++ indicates very severe dermatitis.

2. The incidence of 'rat pellagra' in relation to light. The results reported in the preceding section suggest that vitamin B_6 which cures the dermatitis characteristic of 'rat pellagra' is not identical with the pellagra preventive vitamin postulated by Goldberger. Evidence was therefore sought which would throw light on the relationship of the dermatitis of 'rat pellagra' to the characteristic dermatitis of human pellagra.

To this end forty-nine rats were divided into two groups, one of which was kept in the light and the other in the dark. The animals of both groups were housed in similar cages and both were given the basal diet described by György. In addition to the basal diet supplied ad libitum each rat in each group was given daily by mouth 10 γ of crystalline vitamin B and twice weekly 20 γ of lactoflavin by mouth. The larger group of thirty-seven rats was kept in a light room with windows on the east, south and west sides. Direct sunshine fell several hours of most days of the summer upon these windows, but the rat cages were sufficiently far from the windows to be out of the direct sunshine. The smaller group of twelve rats was kept in a photographic darkroom protected by a maze so that daylight did not penetrate it. A very dim artificial light was maintained throughout the period of experiment by keeping a shaded 40 watt electric lamp burning; arranged in such a way that the light was all thrown on to a small area of the wall away from the neighborhood of the animals. This supplied a general intensity of illumination just great enough to enable one to see one's way about. An overhead electric lamp supplying a stronger light was used while feeding, dosing or weighing the rats.

The incidence of rat 'pellagra' among the rats of the two groups was found to be similar as can be seen from table 2 where the data are summarized. A similar proportion of rats developed the dermatitis in each of the two groups and the average time on the deficient diet before symptoms appeared was almost the same for each group. In each group a number of the rats remained free from dermatitis and grew through-

out the period of feeding on the B_6 deficient diet; an example of refection.

3. Clinical experience with maize meal and with lactoflavin. By the courtesy of Dr. David T. Smith and Dr. J. M. Ruffin of the department of medicine, Duke University, I am able to include the following case reports made by them upon patients seen in Duke Hospital. Ruffin and Smith ('34) have elaborated a fully controlled technic for testing the pellagra-curative action of foodstuffs and special preparations. The patient on entering the hospital is placed on a diet which is arranged to be deficient in vitamin G but satisfactory in all other respects. After 4 or 5 days the patient's sensitivity toward light is determined and if it is found to be super-

TABLE 2

The effect of illumination on the incidence of rat dermatitis

GROUP	TOTAL RATS IN GROUP	ILLUMINATION OF QUARTERS	RATS DYING WITHOUT DERMATITIS	RATS SHOWING REFECTION	RATS DEVELOPING DERMATITIS	AVERAGE NUMBER OF DAYS ON DEFICIENT DIET BEFORE DERMATITIS DEVELOPED
1	37	Strong daylight	4	6	27	96 (standard deviation 11.7)
2	12	Darkroom	1	2	9	98 (standard deviation 11.3)

normal (characteristic of pellagra) the food or preparation to be tested for pellagra-curative activity is added to the basic diet. If the material is active, then the patient will show rapid improvement and after the lapse of 7 to 10 days his light sensitivity is again examined. If it has returned to normal, then a clear-cut case showing the activity of the material under test will have been obtained. But if the material is inactive, the patient's condition will not improve, and his light-sensitivity on re-examination will again be supernormal. He is then given a known pellagra curative (Valentine's Liver Extract) and if his recovery follows during the ensuing 7 to 10 days and his light sensitivity returns to normal, clear-cut evidence showing the inactivity of the test material will have been obtained.

By means of this technic it is possible to determine finally the pellagra-curative activity of a dietary supplement by means of a few cases, provided that the routine of the test can be rigorously adhered to. It frequently happens, however that the routine must be modified in order to avoid danger to the life of the patient or for other reasons, and the result of the test can then only be taken as an indication, but not as proof positive of the activity or inactivity of the test material.

TABLE 3

Standard basic diet, no. 2

ARTICLE	QUANTITY	PROTEIN, GRAMS	FAT, GRAMS	CARBOHYDRATE, GRAMS	MINERALS, GRAMS			CALORIES
					Ca	P	Fe	
Corn meal	92 gm.	8.3	2.0	69.0	0.011	0.1225	0.0006	2890.0
Cane syrup	105 gm.	....	....	89.2	....	....	....	
Flour	111 gm.	12.5	1.2	83.4	0.022	0.1030	0.0010	
Lard	81 gm.	....	81.0	....	....	....	....	
Rice	25 gm.	2.0	0.1	19.6	0.0023	0.0240	0.0002	
Field peas	90 gm.	19.2	1.4	54.6	0.0756	0.0760	0.0052	
Hominy grits	51 gm.	4.3	0.3	40.6	0.0056	0.0734	0.0005	
Fat salt pork	60 gm.	1.1	51.3	0.0	0.0011	0.0115	0.0001	
Cod liver oil	90 ml.	....	80.0		....	0.0117	....	720.0
Ascorbic acid	90 mg.	0	0		0	....	....	
Iron ammonium citrate	6 gm.	0	0		0	0	1.02	
Calcium gluconate	6 gm.	0	0		0.5580	0	0	
Cheese	60 gm.	17.4	21.6		0.5586	0.4098	0.0007	264.0
Total		64.8	238.9	356.40	1.2387	0.8319	1.0283	3874.0

The basic diet used is constituted as shown in table 3. It will be seen that it contains 92 gm. of maize meal (white) and 51 gm. of hominy grits. Thus the patient receives 143 gm. daily of white maize in these two forms and this exerts no curative effect on the pellagra. Three pellagrins were given

treatment with lactoflavin during the summer of 1935 as described below:

Case 1. (No. 53,890 S. T. A colored female, age 34, was admitted to the hospital June 12, 1935, presenting a typical picture of pellagra, with a rash over the hands, a sore tongue and diarrhea of 4 weeks' duration. She was given the Standard Basic Diet no. 2 (shown in table 3) and commencing on the day after admission a solution of lactoflavin containing 0.5 mg. per ml. was administered subcutaneously, 2 ml. the first day, 8 ml. the second and 4 ml. daily thereafter for 6 days. During this time she failed to improve; the anorexia continued; the diarrhea and tongue became much worse. As no improvement was observed after 8 days of this treatment the lactoflavin was discontinued and the patient was given daily 90 cc. by mouth of Valentine's Liver Extract which had been shown previously by Ruffin and Smith ('34) to be effective in the treatment of pellagra. After 3 days she began to improve and within 5 days she was apparently entirely well.

Case 2. (No. 53,398) J. C. A white male, age 39, was admitted to the hospital June 3, 1935, with a marked diarrhea, a rash over both hands, and a red beefy tongue. He was somewhat disoriented. He was given the Standard Basic Diet no. 2 and observed over a period of 5 days. During this time the tongue remained the same and the diarrhea continued unabated.

Beginning June 7th, 15 ml. of a preparation of liver (no. 266—a fraction of liver which subsequent studies have indicated to be of little or no value in the treatment of pellagra) was given subcutaneously daily for 10 days. After the second day the tongue was definitely better but the diarrhea was unaffected. Since only slight improvement in the general condition was noted after 5 days, the treatment was supplemented by 8 ml. of lactoflavin daily, administered subcutaneously, for 7 days. No definite change was noted at the end of this period.

On June 17th, the right hand and arm were exposed to direct sunlight for 30 minutes and for 45 minutes the following day. No rash was observed but the tongue became very red and sore. The lactoflavin and liver fraction (no. 266) were discontinued and the patient given 90 ml. of Valentine's Liver Extract by mouth. The tongue presented a normal appearance after 4 days of this treatment and there was noted an improvement in his general condition although the diarrhea remained unchanged at time of his discharge, 7 days after instituting the liver therapy by mouth.

Case 3. (No. 31,387). A white woman, age 43, was admitted to the hospital on May 21, 1935. On a previous admission, she had been found to have amyotrophic lateral sclerosis. Her present illness began 4 weeks before admission with a rash over the hands and face and diarrhea following exposure to sunlight.

She was given the Standard Basic Diet no. 2 and observed for a period of 7 days, during which time her diarrhea became worse. On the eighth and ninth days (May 27th and 28th) her right arm and hand were exposed to direct sunlight for 30 and 60 minutes, respectively. Immediately following this she developed nausea and vomiting; the tongue became worse and the diarrhea increased. Lactoflavin, 4 ml. subcutaneously, was given daily for the next 3 days during which time she became progressively worse. On June 1, it was apparent that she was critically ill and was given large amounts of active liver extract by mouth and glucose intravenously. The patient did not respond to treatment and died on June 2nd.

DISCUSSION

Perhaps the most significant of the observations recorded above is the finding that dermatitis can be induced in rats kept in darkened rooms and given a vitamin B_6 deficient diet. Examination of the figures showing incidence of the dermatitis among the rats kept in a darkened room and those kept in strong summer daylight suggests at once that light plays no part in the production of the lesions; and this conclusion is supported by the observation that among the rats developing dermatitis the average time on the deficient diet before the dermatitis appeared was almost the same for the two groups.

On the other hand the clinical experience of Ruffin and Smith ('35) has shown that the dermatitis of the exposed surfaces of human pellagrins is closely dependent upon the action of sunlight on the patient's skin. Thus there is a sharp distinction between the immediate cause of the dermatitis of the rat and the cause of the dermatitis of human pellagra. A dietary deficiency apparently leads immediately to the production of dermatitis in the rat, but in the human the dermatitis is only produced by the agency of light after

dietary deficiency has played its part. This constitutes an important dissimilarity between the rat dermatitis and human pellagra and affords another reason for reviewing with caution past attempts at tracking down the pellagra preventive factor by means of rat feeding experiments. Since much of the past work was done under the assumptions that vitamin G is a single entity and that the rat dermatitis is strictly analogous to human pellagra, its conclusions may be invalidated by the demonstration that these assumptions are untenable. It must, however, be borne in mind that although this difference between the dermatitis of the two species has been demonstrated, the underlying dietary deficiency in pellagra may yet be identical with the deficiency which directly produces rat dermatitis.

Suggestive but inconclusive evidence on this point is afforded by the observations recorded under part 1 of the experimental section, which show that a daily dose of 1 gm. of either the white or the yellow maize meal employed is sufficient to cure rat dermatitis. As the white maize meal was taken from the stock used in preparing diets for patients in the hospital, this observation appears to suggest that the substance (vitamin B_6) which cures rat dermatitis is not identical with the pellagra-preventive factor of Goldberger. This conclusion was considered highly probable by Birch and György ('35) who found that 0.5 gm. of maize meal was the minimum daily dose needed to cure rat dermatitis.

Turning now to the clinical evidence, it is to be noted that the Standard Basic Diet no. 2 contains 143 gm. of maize (92 gm. of meal and 51 gm. of grits); i.e., 286 times the minimum dose for the cure of rat dermatitis. The approximate ratio of weights of the human being and rat is about 600:1, so that weight for weight the patients received in the basic diet about half as much vitamin B_6 as the rats were given. At this level of feeding no improvement at all was seen in their condition. The amount of maize eaten by these patients during the development of their pellagra is likely to have been greater than that contained in Standard Basic Diet no. 2. Moreover,

Goldberger and Wheeler ('20) produced human pellagra experimentally by feeding to their subjects a diet containing about 250 gm. of maize daily or 500 rat day-doses of vitamin B_6. Therefore it is clear that 1) vitamin B_6 is a different entity from the pellagra-preventive factor, or 2) vitamin B_6 and the pellagra-preventive factor are identical, but the human requirement of this factor relative to weight is much greater than the rat's, or 3) either vitamin B_6 or the pellagra-preventive factor is a complex of which the other is one component. The weight of evidence appears at present to be in favor of the first alternative.

The clinical observations with lactoflavin again are suggestive but not final. In case I the patient received 17 mg. of lactoflavin over a period of 8 days, an amount corresponding to a daily average of 300 oral day-doses for a rat. This dosage given subcutaneously was considered to be substantially similar, relative to weight, to the minimum needed by the rat, but no improvement resulted. Replacement of the lactoflavin by Valentine's Liver Extract led to improvement in 3 days. In case 2 the patient received 4 mg. daily of lactoflavin over a period of 5 days and showed no definite improvement. He subsequently improved rapidly when given the potent liver preparation, but left the hospital with one symptom persisting. In case 3 the patient deteriorated in condition while receiving lactoflavin and then suddenly became worse and died. Thus no one of the three cases was carried through the full routine of the test as described above, and the evidence afforded by them is not final. It is indefinite but suggests that lactoflavin has no pellagra-curative activity. A further study of lactoflavin will be made during the pellagra season of 1936.

SUMMARY

1. Rat dermatitis appears among rats on a B_6 deficient diet as readily in the dark as in the light, and is therefore not analogous to the dermatitis of human pellagra.

2. One gram of white or yellow maize meal contains at least one 'curative day-dose' of vitamin B_6, suggesting that vitamin B_6 is not identical with the pellagra-preventive factor.

3. Preliminary clinical trials with lactoflavin suggest that lactoflavin possesses no pellagra curative activity.

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THE EFFECT OF FEEDING EGG YOLK ON THE LIVER LIPIDS OF YOUNG RATS

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That the ingestion of lecithin may partially or wholly prevent the development of fatty livers by rats fed diets high in fat has been indicated by the studies of Best and Hershey ('32). The same group of investigators have reported that choline is the component of the lecithin to which this action may be ascribed (Best and Huntsman, '32; Best and Ridout, '33). Blatherwick and his co-workers ('33) were, however, able to demonstrate no effect on the liver lipids of rats fed egg yolk and liver diets when lecithin was given.

Channon and Wilkinson ('35) and Aylward, Channon and Wilkinson ('35), since the present investigation was well under way, have presented figures which indicate that, while choline does affect the deposition of fat in the livers of rats on a high fat diet, it has a much less marked, if appreciable, influence on the deposition of cholesterol ester.

Best's et al. ('35) most recently published results are again not entirely in agreement with Channon's.

From the point of view of practical application in dietetics the need for further investigation of the effect of lecithin on reaction to high cholesterol diets seems self evident. Almost all cholesterol rich foods are at the same time excellent sources of lecithin—e.g., egg yolk, brains, liver. In our own laboratory at a comparatively low level of cholesterol intake, the effect of egg yolk feeding on the blood cholesterol of normal women was quite comparable to that observed when the same women took cholesterol with fat alone (Okey and Stewart, '33).

The increase in the incidence of arteriosclerosis and gall bladder disease, especially among diabetics, is hard to explain without taking into consideration the possible effect that the use of large numbers of eggs in the dietary may have. A recent reviewer (Brody, '35) comments, "only humans die in early life from coronary sclerosis, only humans suffer almost universally from atherosclerosis with advance of age, only humans consume regularly during adult life cholesterol rich foods."

Nevertheless in one of the recent textbooks in dietetics Newburgh and McKinnon ('34) propose no less than eight out of ten skeleton therapeutic diets which call for the use of from five to seven eggs per person per day. Certainly a sufficiently large number of people survive dietary regimes in which eggs are used habitually to suggest that it may be to the point to investigate the results of egg consumption when the other constituents of the dietary are carefully controlled. Our present study has therefore dealt with the effect of egg yolk feeding on the liver lipids of rats on diets which are, in so far as can be determined, adequate to meet all nutritional requirements.

EXPERIMENTAL

The diets were made up to contain egg yolk, EYC, egg yolk protein plus neutral fat plus cholesterol, ECC, and egg yolk protein plus fat alone, EYF. (For details of composition see table 1.) Individual cages with 'safety' food cups were used, and the respective diets given ad libitum.

At the close of the experimental period the rats were killed, the tissues ground, and the alcohol-ether extracts prepared and analyzed as described in a previous paper (Okey, Gillum and Yokela, '34). Cholesterol was in all cases determined both colorimetrically and by digitonid precipitation, but for the sake of consistency with the other lipid analyses all figures given are for data from the microxidation procedure. Individual data are given in table 2 and summarized in table 3.

It will be seen that the livers of the egg yolk-fed animals contained cholesterol ester in quantities which, while appreciably less than those observed in the animals fed cholesterol with fat, still averaged more than twelve times the concentration in the controls. These livers were whitish, and presented much the same appearance of gross engorgement with lipid presented by those of the cholesterol fed controls.

Cholesterol intake and cholesterol storage in the liver. The growth of cholesterol fed animals will be discussed in a separate paper. It is necessary to point out, however, that the

TABLE 1

Composition of experimental diets

	DIET [1] EYC PARTS	DIET ECC PARTS	DIET EYF PARTS
Commercially dried egg yolk[2]	25.3	...	...
Alcohol-ether extracted egg yolk	...	8.4	8.4
Extracted casein	11.6	11.6	11.6
Agar	4.0	4.0	4.0
Salt (Osborne-Mendel)	3.0	3.0	3.0
Starch	58.1	58.0	58.0
Crisco	...	15.0	15.0
Cholesterol	...	1.0	...
Total lipid	16.0	16.0	15.0
Total protein	17.5	17.7	17.7
Calories per gram; diet (excluding cholesterol)	4.4	4.5	4.5

[1] Vitamin supplements: Yeast, 0.5 gm. rat/day; CLO, 2 drops/rat/day.

[2] Composition: Protein (N × 6.25) 29.2 per cent, alcohol-ether ext. 63.8 per cent, lecithin 9.6 per cent, ash 3.3 per cent, cholesterol 3.9 per cent.

growth of both egg yolk-fed and cholesterol-fed controls compared very favorably with that of animals on a very good stock diet.

The experimental diets were planned to furnish as nearly an equal number of calories per gram food as possible. Nevertheless, the egg yolk-fed animals ate so much less per unit gain in weight that in the animals fed only 60 days the cholesterol intake of the cholesterol-fed control group (ECC) per 100 gm. body weight was about 1.4 times that of the group fed the egg yolk. The rats fed cholesterol as such show liver

TABLE 2

Liver lipids of rats

NO.	SEX	WEIGHT RAT GRAMS	WEIGHT LIVER GRAMS	MOISTURE, PER CENT	FATTY ACID	TOTAL CHOL.	ESTER CHOL.	FREE CHOL.	LECITHIN	LIVER CHOL. MG. RAT
					Per cent moist weight liver					
Diet E.Y.C.—60 days										
59	♂	325	14.9	60.3	12.5	3.00	2.77	0.23	3.1	448
61	♂	253	8.8	62.7	8.8	2.45	2.15	0.30	2.9	215
68	♂	265	9.1	57.7	13.5	2.84	2.51	0.33	3.2	251
71	♂	289	10.3	64.1	7.8	2.23	1.95	0.28	2.8	230
P(3)[1]	♂	196	9.7	63.8	9.3	2.58	2.31	0.27	2.7	250
69	♀	188	7.2	60.3	12.6	4.05	3.76	0.29	2.7	293
70	♀	160	6.4	...	7.7	2.19	1.79	0.40	3.0	136
72	♀	176	5.8	61.4	11.5	4.10	3.73	0.37	2.7	232
73	♀	165	6.2	59.6	12.0	6.50	6.20	0.33	2.6	403
P(3)[1]	♀	172	6.1	62.4	11.7	3.86	3.52	0.34	2.5	237
Diet E.Y.C.—120 days										
58	♂	316	9.2	68.0	5.6	1.13	0.89	0.24	2.5	104
60	♂	325	9.0	63.0	5.7	2.46	2.12	0.34	2.8	224
66	♂	356	10.9	60.9	5.7	5.33	4.97	0.38	2.6	580
67	♂	361	11.8	64.6	7.6	2.46	2.06	0.40	2.8	280
62	♀	225	7.6	56.2	16.7	3.50	3.26	0.24	2.3	266
63	♀	210	7.8	59.0	14.8	3.56	3.25	0.31	1.5	278
64	♀	238	8.5	55.0	20.5	5.12	4.32	0.38	2.2	436
65	♀	210	7.5	52.6	19.6	5.75	5.45	0.30	2.0	434
Diet E.C.C.—60 days										
04	♂	213	11.4	48.4	25.6	7.15	6.92	0.23	2.8	810
07	♂	224	9.1	55.4	18.9	4.85	4.63	0.22	3.2	443
10	♂	251	11.9	56.1	17.8	4.50	4.30	0.20	3.0	534
12	♂	203	9.7	...	19.3	7.35	7.12	0.23	3.0	710
03	♀	148	8.2	57.8	17.9	3.46	3.10	0.36	2.1	620
06	♀	162	7.7	58.0	17.6	5.50	4.14	0.36	2.6	425
08	♀	145	8.2	51.9	20.1	8.20	7.82	0.38	1.8	677
09	♀	143	7.8	57.8	17.1	7.20	6.83	0.37	1.9	560
Diet E.C.C.—120 days										
02	♂	350	14.6	61.0	10.9	4.30	4.15	0.15	2.5	627
05	♂	295	15.7	51.6	21.0	6.20	6.04	0.15	2.2	970
11	♀	225	8.9	64.8	11.5	2.60	2.50	0.10	2.7	260
13	♀	243	10.4	58.4	16.7	5.45	5.32	0.13	2.3	570
Diet E.Y.F.—60 days										
14	♂	220	6.9	70.3	3.8	0.25	0.02	0.23	2.0	17
49	♂	203	6.8	63.3	4.9	0.29	0.06	0.23	1.9	20
52	♂	210	7.1	67.6	5.9	0.29	0.01	0.28	2.4	21
53	♂	207	7.0	70.0	4.5	0.29	0.01	0.28	2.5	20
P(6)[1]	♂	208	8.3	65.9	7.5	0.48	0.18	0.30	2.7	40
16	♀	190	6.9	67.1	8.1	0.27	0.03	0.24	2.3	18
17	♀	178	6.6	67.8	6.5	0.22	0.00	0.22	2.2	13
50	♀	154	5.1	67.7	7.2	0.24	0.00	0.25	2.5	13
51	♀	154	6.0	67.8	5.1	0.24	0.01	0.24	2.6	15
P(6)[1]	♀	152	8.0	66.1	7.4	0.30	0.01	0.29	2.0	20
Diet E.Y.F.—120 days										
15	♂	285	8.8	65.4	7.9	0.33	0.13	0.20	2.6	29
55	♂	253	7.3	68.5	5.1	0.28	0.11	0.17	2.7	20
48	♀	190	5.6	73.4	7.3	0.29	0.04	0.25	2.3	16
54	♀	196	5.7	...	7.5	0.28	0.07	0.21	2.0	20

[1] Pooled samples from the number of rats indicated.

TABLE 3

Summary of data. Determinations for individual animals; 60- and 120-day groups taken together. Equal numbers of males

TABLE 3

Summary of data. Determinations for individual animals: 60- and 120-day groups taken together. Equal numbers of males and females in each group

	EYC	ECC	EYF	DIFFERENCES		
				EYC and ECC	EYC and EYF	ECC and EYF
Weight rat grams	254 ±11.8	217 ± 12.2	203 ±6.9	37 ± 17	51 ±13.7	14 ±14
Weight liver grams	8.8 ± 0.39	10.3 ± 0.51	6.6 ±0.20	1.5 ± 0.20	2.2 ± 0.44	3.7 ± 0.55
Total F.A. per cent moist weight	11.4 ± 0.81	17.9 ± 0.76	6.1 ±0.28	6.5 ± 1.11	5.2 ± 0.86	11.7 ± 0.8
Total chol. per cent moist weight	3.5 ± 0.25	5.6 ± 0.27	0.27±0.01	2.0 ± 0.37	3.26± 25	5.3 ± 0.27
Ester chol. per cent moist weight	3.2 ± 0.25	5.2 ± 0.30	0.05±2.0	2.0 ± 0.38	3.15± 25	5.2 ± 0.30
Free chol. per cent moist weight	0.32± 0.01	0.24± 0.02	0.23±0.01	0.08± 0.023	0.09± 0.014	0.01± 0.023
Lecithin per cent moist weight	2.62± 0.07	2.52± 08	2.33±0.10	1.10± 0.111	0.29± 0.16	0.19± 0.16
Total liver chol. mg./rat	301 ±17.7	601 ±125	18.6 ±0.86	300 ±126	282 ±17.7	582 ± 0.9

EYC = egg yolk diet.
ECC = extracted egg yolk with cholesterol and Crisco.
EYF = extracted egg yolk with Crisco only.

cholesterol and fatty acid values consistently about 1.5 to 2 times those of the egg yolk-fed animals. At the end of the period of rapid growth the differences in food intake were very much less marked, and this was also true of the differences in cholesterol ester concentration in the liver, the respective mean being, cholesterol-fed group 4.50 per cent and the egg yolk-fed group 3.29 per cent.

The livers of the egg yolk-fed and control animals did not continue to enlarge as they grew older, but the small group fed cholesterol show a rather great increase in size. This suggests that the continued enlargement of the liver with age accompanied by increased storage of cholesterol ester is the chief defense mechanism against cholesterol invasion of other tissues in the rat fed cholesterol without lecithin. The egg yolk-fed animals, on the other hand, had no larger livers at 120 than at 60 days, and only very little more liver cholesterol per rat. This must indicate that their cholesterol is either moved to other tissues and deposited or that it is destroyed.

Effect of fat intake and age of animals

Cholesterol feeding. In order to utilize egg yolk powder to furnish cholesterol at a 1 per cent level, the total lipid concentration of the diet other than cholesterol was automatically raised to 16 per cent. Comparison with our figures for control animals fed 5 and 10 per cent fat shows that this in itself apparently had no effect on the fatty acid concentration of the livers. That of the cholesterol-fed animals was approximately 2 per cent greater (mean 19.3 per cent) than the mean of our previous observations on animals fed 5 and 10 per cent fat in the diet. Cholesterol ester concentrations varied only within the limits to be expected to be accounted for by chance in the 60-day control groups, i.e., for animals 83 to 85 days old.

Both fatty acid and cholesterol were lowered in the livers of the animals fed cholesterol for 120 days and varied hardly at all in the fat fed controls.

Egg yolk feeding. In the case of the animals fed egg yolk, however, there seemed to be a tendency for the fatty acid

concentration of the livers of the males to decrease and of the females to increase with age.

The series fed egg yolk for 120 days was not large, but the fact that the average liver fatty acid concentration in the females fed egg yolk for 120 days was almost three times that of the males, and one and three-quarters that of the females fed only 60 days, is at least interesting. Why the fatty acid concentration in the livers of the males fed egg yolk should decrease with age while that of the females increases is hard to explain as is the reason for the very much lower concentration of cholesterol ester in the livers of the male egg yolk-fed rats of both age groups.

If we assume that the holding back of cholesterol as ester by the liver is a defense mechanism designed to prevent its delivery to and accumulation in the tissues in which its presence is most dangerous to life processes, such for instance as the intima of the coronary arteries, we may correlate this sex difference in the response of the liver to continued ingestion of egg yolk diets with the higher incidence of arteriosclerosis in men, and of diseases associated with hepatic function in fat and cholesterol metabolism in women, e.g., gall stones.

Certainly the differences between the cholesterol-fed controls and the egg yolk-fed rats indicate some sex variation in the part played by the lecithin. In view of Best's findings it is especially interesting that the egg yolk-fed females had higher liver fatty acid values than the animals of either sex fed cholesterol without lecithin. Likewise the egg yolk-fed animals of both sexes had slightly higher total liver cholesterols at 120 days than at 60 days, a tendency apparently more manifest even than that of animals fed cholesterol without lecithin to increase liver cholesterol concentration with age.

These findings have led us to undertake to study the effect on the distribution of cholesterol in the tissues of feeding cholesterol and egg yolk diets during the entire life span of the rat. Further discussion is postponed until this can be done.

SUMMARY AND CONCLUSIONS

Rats fed diets containing egg yolk to furnish 1 per cent cholesterol tend to develop fatty livers in spite of the 2.3 per cent phospholipid content of this diet. The accumulation of fat and cholesterol ester tends, in females fed for 120 days, to be even greater than in animals fed egg yolk protein and cholesterol at the same level with hydrogenated vegetable oil[1] to replace the egg yolk lipid and no lecithin. Males tend to store less liver fat and cholesterol on egg yolk than on cholesterol diets.

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[1] Crisco.

THE COMPARATIVE EFFECTS OF COD LIVER OIL, COD LIVER OIL CONCENTRATE, LARD AND COTTONSEED OIL IN A SYNTHETIC DIET ON THE DEVELOPMENT OF NUTRITIONAL MUSCULAR DYSTROPHY

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TWO PLATES (EIGHT FIGURES)

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When some herbivorous animals, namely, guinea pigs and rabbits (Goettsch and Pappenheimer, '31; Woodward and McCay, '32), as well as goats and sheep (Madsen, McCay and Maynard, '33, '35) are fed certain synthetic and semi-purified rations containing the known dietary essentials, nutritional failure usually results. A prominent cause of death on such rations is the development of a progressive, highly specific degeneration of the skeletal muscles.

During experimental inquiry into the fundamental food requirements of Herbivora by means of the synthetic diet technic results were obtained by Madsen, McCay and Maynard ('33, '35) which indicated that addition of cod liver oil to the basal ration increased the rate of development and early severity of the muscle degeneration. The substitution of a cod liver oil concentrate in the synthetic ration greatly delayed the production of muscle lesions and allowed longer survival and more growth, but did not prevent the eventual development of muscle dystrophy. Moreover, the entire elimination of cod liver oil from the synthetic diet and the use of carotene and irradiated yeast as a source of the A and D vitamins did not prevent the development of the muscle degeneration. Muscle lesions were also produced in guinea

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pigs fed a stock diet supplemented with cod liver oil, but the natural foods supplied considerable protection against the development of lesions. These results indicated that both cod liver oil and other factors of the synthetic diet affected the production of muscle dystrophy in the animals studied.

This report deals with experiments on the production of muscle dystrophy which were planned to define more clearly the effect of cod liver oil and a vitamin A-D concentrate of cod liver oil as supplements to rations containing only lard or cottonseed oil as the main source of fat. These comparisons were made principally with a synthetic diet and also with a modification of diet 11 used by Goettsch and Pappenheimer ('31). The effect of a diet of grains with and without a supplement of cod liver oil was also studied with guinea pigs.

The experimental animals were killed at intervals or were examined at death after being on the diets for varying lengths of time. The presence of muscle changes was determined, by gross and histopathological examination. In many cases oxygen consumption and creatine determinations[2] were made on the excised muscle tissue. The data obtained with guinea pigs and rabbits are considered together, since the experiments and results were largely comparable.

EXPERIMENTAL WORK

Methods. Young guinea pigs weighing from 202 to 380 gm. and rabbits weighing between 520 and 1440 gm. were changed gradually from a normal stock diet to a basal synthetic ration of the following general composition:

	Per cent
Purified cellulose (sylphrap)	20
Casein	15
Sucrose	10
Starch	43 (or less)
Fat (lard or cottonseed oil)	3 (or more)
Yeast	5
Mineral mixture (Hawk-Oser, '31)	4
	100

[2] I am indebted to Dr. Marianne Goettsch for the creatine determinations included in this report.

Seven groups of guinea pigs and six groups of rabbits were fed this basal ration with variables as to the source and amount of fat and the source of vitamins A and D. The diets used in this study are summarized in table 1.

The guinea pigs and rabbits of group A were fed the basal synthetic ration containing either lard or cottonseed oil supplemented with cod liver oil. In rations A-2, A-3 and A-4,

TABLE 1

Diets fed to guinea pigs and rabbits

GROUP	BASAL DIET	SOURCE OF FAT	SOURCE OF VITAMINS A AND D	EXPERIMENTAL ANIMALS	
				Guinea pigs	Rabbits
A-1	Synthetic	3% lard	0.5 gm. CLO daily	5	..
2	Synthetic	4% lard	2.0% CLO	..	8
3	Synthetic	3 and 6% CSO[1]	0.5 gm. CLO daily	10	..
4	Synthetic	4 and 8% CSO	2.0% CLO	..	6
B-1	Synthetic	6% lard	0.05% A-D concentrate[2]	..	2
2	Synthetic	3% CSO	8 mg. A-D concentrate in 0.5 gm. CSO daily	10	..
3	Synthetic	6% CSO	0.05 % A-D-concentrate	..	6
C-1	Synthetic	None added	0.05% A-D concentrate	5	3
2	Synthetic	4% Sap. CLO	0.05% A-D concentrate	5	..
3	Synthetic	4% Sap.CSO	0.05% A-D concentrate	5	..
D-1	(Modified	3.2% lard	0.5 gm. CLO daily	5	..
2	diet 11)	3.2% lard	8 mg. A-D concentrate in 0.5 gm. CSO daily	5	..
E-1	Grains	.....	.....	10	..
2	Grains	.....	0.5 gm. CLO daily	5	..
F-1	Normal	.....	.....	5	4

[1] Cottonseed oil fed as Wesson oil.

[2] The A-D concentrate of cod liver oil was kindly furnished by Dr. T. F. Zucker.

where more than 3 per cent of either lard or cottonseed oil was included, the composition of the diet was adjusted by decreasing the percentage of starch. The cod liver oil fed to guinea pigs of groups A-1 and A-3 was emulsified in a daily allowance of 7 to 10 cc. of tomato juice containing 0.2 gm. dried yeast. The cod liver oil supplement for the rabbits of groups A-2 and A-4 was included in the ration on a percentage basis. No source of antiscorbutic vitamin was provided in the rabbit diets.

The synthetic diets fed to rabbits of group B were supplemented with 0.05 per cent of A-D concentrate and the lard (group B-1) and cottonseed oil (group B-3) were increased to 6 per cent to equalize the total fat content of the rations. The guinea pigs of group B-2 were supplied with 3 per cent of cottonseed oil in the basal ration and the A-D concentrate was fed in a solution of 0.5 gm. cottonseed oil daily which was emulsified with yeast and tomato juice as previously described.

Previous experiments by Madsen, McCay and Maynard ('35) have shown that guinea pigs fed a synthetic diet containing lard and the saponifiable fraction of cod liver oil plus carotene and irradiated yeast as the source of the vitamins A and D developed a severe muscular degeneration. This indicated that a ration containing cod liver oil or its saponifiable fraction resulted in the development of muscle changes of a similar character. However, later work with rabbits during the present study suggested that the fat component of the diet should be considered as a possible variable in the production of muscle lesions. This relation was studied further by feeding the synthetic diet without added fat as a control diet (group C-1) and with the addition of the saponifiable fraction of cod liver oil (group C-2) and of cottonseed oil (group C-3) to the basal synthetic diet. These rations were supplemented with the A-D concentrate.

Two groups of guinea pigs were used to compare the use of cod liver oil (group D-1) and the A-D concentrate in cottonseed oil solution (group D-2) as a supplement to diet 11 which has been shown by Goettsch and Pappenheimer ('31) to produce muscle dystrophy in rabbits and guinea pigs. Diet 11 was modified by reducing the lard and it contained the following ingredients:

Rolled oats	355	Lard	30
Wheat bran	180	NaCl	10
Skim-milk powder	275	$CaCO_3$	15
Casein	75		

Previous work in this laboratory by Goettsch and Pappenheimer (unpublished data) has shown that if rabbits are fed

a diet consisting of grains alone a type of degeneration of the skeletal muscles will develop. This grain mixture which consisted of oats 65, barley 85, wheat 45 and wheat bran 5 was finely ground and fed to guinea pigs without (group E-1) and with (group E-2) a supplement of cod liver oil.

Animals of group F-1 were normal controls and received a stock ration which consisted of grains as fed to group E with the addition of alfalfa hay and lettuce.

The pathology and histology of nutritional muscle dystrophy has been described by Goettsch and Pappenheimer ('31) and Madsen, McCay and Maynard ('35). Some advanced stages of muscle degeneration are shown in plate 1. A detailed histological examination of several muscles from each experimental animal is essential, since a single muscle may or may not prove to be a reliable index as to the extent of the muscle lesions. The characteristic symptoms of muscle involvement are not usually obvious in the initial stages of the disease, however, advanced changes can readily be detected.

Routine muscle samples for histological examination were taken from the quadriceps, gastrocnemius, semitendinosus, adductor of the thigh, triceps, biceps, pectoralis and heart muscle. Occasionally, samples were taken from the masseter, tongue, diaphram, intercostals, soleus, and eye muscles. For purposes of tabulation the extent of the muscle lesions are graded from slight one-plus degeneration to the more severe two-, three- and four-plus changes as shown in plate 2 of a previous publication (Madsen, McCay and Maynard, '35).

Various organ samples were also taken for histological study. The liver of the dystrophic guinea pig is often larger than normal. The kidneys of both rabbits and guinea pigs were occasionally mottled, pitted and scarred. Cases of the so-called spontaneous medial calcification of the aorta were seen rather frequently in rabbits. A terminal bronchopneumonia developed in some advanced cases. Skeletal muscle dystrophy frequently occurs in cases apparently free from complicating diseases which seems to emphasize that the disease is highly selective and specific of skeletal muscle.

Some of the guinea pigs were killed at approximately 10-day intervals for histological examination and for oxygen consumption and creatine determinations on the skeletal muscles. Other animals were allowed to continue for longer periods on their respective diets. At intervals, under ethyl ether anesthesia, pieces of adductor muscle of the thigh were excised from some of the surviving guinea pigs and rabbits for respiration studies and histological examination.

The oxygen consumption determinations were made by the use of Fenn volumeters after the method outlined by Victor ('34). The value recorded as the oxygen consumption represents the average cubic millimeters of oxygen consumed per gram of moist tissue per minute during the second hour of the experiment. The muscle tissue was cut with scissors into fine longitudinal strips and a weighed sample of 75 to 200 mg. was placed into Ringer's solution containing a phosphate buffer of pH 7.4. The respirometers were flushed out with pure oxygen and then immersed and shaken in a water bath at a temperature of 37.5 $\pm$ 0.005°C. The oxygen consumption was followed for varying lengths of time between 2 and 8 hours.

The presence of muscle degeneration can usually be detected histologically in the portion of muscle used in the respiration determination, but the extent of the muscle change may be somewhat confusing, since the sarcoplasm may be altered during the experiment. In the case of rabbits, where plenty of tissue is available, some of the original biopsy material was fixed in Zenker's solution immediately and the remaining portion was used for the oxygen consumption determination and then fixed for microscopic study. Both samples were embedded together for sectioning and then mounted and stained in the usual manner.

Creatine was determined colorimetrically in muscle tissue as described by Goettsch and Brown ('32), and the comparisons were made with the use of a photo-electric colorimeter.

Results. The incidence of muscular lesions in the animals fed the various experimental diets will be given first and will

be followed by a presentation of the results of the oxygen consumption and creatine determinations.

The data for the rabbits and guinea pigs fed the synthetic diet containing either lard or cottonseed oil and supplemented with cod liver oil are summarized in tables 2 and 3. The animals developed muscle dystrophy very rapidly when fed these diets while the guinea pigs on the normal diet, as usual, failed to develop muscular lesions as shown in table 2. Slightly greater growth and longer survival resulted in several cases when the animals were fed the synthetic diet containing cottonseed oil as the source of fat as compared to lard; however, muscle dystrophy occurred in the animals after about the same interval on both of these rations which were supplemented with cod liver oil as the source of vitamins A and D.

Degenerative changes in the heart muscle consisting of necrosis of muscle fibers, cellular infiltration, calcification and fibrosis were noted in rabbits nos. 3, 14, 5 and 2 (plate 2) and also in rabbits 13 and 15. These areas of degeneration were evident in the gross. In rabbit no. 2 the heart lesion was primarily a vascular one and consisted of intimal thickening and fragmentation of the inner elastic membrane of the arterial walls. There was some fibrosis present in the myocardium in the region of the altered vessels. Heart involvement is not a consistent finding in rabbits or guinea pigs with severe skeletal muscle degeneration.

A summary of the data obtained when the basal synthetic diet was supplemented with an A-D concentrate of cod liver oil instead of cod liver oil are given in table 4.

The two rabbits fed the synthetic diet in which lard provided the main source of fat (group B-1) developed advanced muscle lesions, but the data obtained with guinea pigs and rabbits fed the synthetic diet containing cottonseed oil as the source of fat and the A-D concentrate as the vitamin supplement (groups B-2 and B-3) show an interesting contrast to the results obtained with the previous rations. Only one guinea pig out of ten in group B-2, no. 191, developed muscle

TABLE 2

Summary of results with guinea pigs and rabbits fed a synthetic diet containing lard as the main source of fat and supplemented with cod liver oil as compared to guinea pigs on the normal diet

GROUP	ANIMAL NO.	DAYS ON DIET	BODY WEIGHT				RESULT	SKELETAL MUSCLE DYSTROPHY	OXYGEN CONSUMPTION C.MM./GM./MIN.	MUSCLE CREATINE[1] MG./100 GM.		
			Initial	Days to maximum	Maximum	Final				Sample	1	2
			gm.		gm.	gm.						
A-1 (guinea pig)	165 ♀	12	256	10	284	266	Normal, killed	—	1.18	Mixed	520	518
	161 ♂	21	240	17	306	218	Died,	+ to ++		Mixed	342	404
	162 ♂	33	330	15	384	367	P+,[2] killed	— to ++	2.44	Mixed	422	383
	164 ♀	49	272	35	386	333	P+++, killed	++++	6.57	Triceps	127	
										Quadriceps	105	99
	163 ♂	56					Weak, biopsy	+++	2.02			
	163 ♂	58					Weak, biopsy	++++	2.05	Quadriceps	73	
	163 ♂	59	202	47	354	256	Died,	++++		Triceps	74	
A-2 (rabbit)	2 ♂	0					Normal, biopsy	—	0.57	Semitendinosus	287	
	2 ♂	14	1130	7	1230	970	P++, died	++++		Gluteus	387	344
										Soleus	219	
	14 ♀	0					Normal, biopsy	—	0.50			
	14 ♀	15					P—, biopsy	—	1.18			
	14 ♀	33					P±, biopsy	++	2.03	Red muscle	268	
	14 ♀	40	1300	17	1610	1270	P+++, killed	++++	2.98	White muscle	260	
	15 ♀	0					Normal, biopsy	—	1.65	Soleus	222	
	15 ♀	15					P—, biopsy	—	2.06	Gastrocnemius	343	
	15 ♀	26	1440	24	1540	1450	P+++, killed	+++	1.47	Adductor	225	445
	5 ♂	0					Normal, biopsy	—	1.63	Gastrocnemius	366	
	5 ♂	15					P—, biopsy	—	1.45	Quadriceps	399	
	5 ♂	17	1060	3	1100	900	P—, died	+++		Soleus	322	
										Semitendinosus	319	
	22 ♀	6	740	1	740	550	Moribund, killed	±				
	23 ♀	31	750	16	860	640	P++++, killed	++++				
	24 ♀	47	740	7	790	450	P+++, died	++++				
	25 ♂	45	950	26	1030	710	P+++, died	++++				
F-1 (guinea pig)	172 ♀	80	250	80	520		Normal, biopsy	—	0.54			
	173 ♀	47	220	47	496	496	Normal, killed	—	1.34	Mixed	416	478
	193 ♂	83					Normal, biopsy	—	1.24			
	193 ♂	208	219	194	920	910	Normal, killed	—	2.72	Mixed	372	
	194 ♂	36	216	36	417	417	Normal, killed	—	1.07	Mixed	399	413
	195 ♂	73					Normal, biopsy	—	0.54			
	195 ♂	209	186	209	800	800	Normal, killed	—	3.12	Mixed	381	

[1] Creatine determinations were made only on muscles at death of animal.
[2] Symptoms of paralysis the extent of which is indicated by plus signs.

Symptoms of paralysis the extent of which is indicated by plus signs.

TABLE 3

Summary of results with guinea pigs and rabbits fed a synthetic diet containing cottonseed oil as the main source of fat and supplemented with cod liver oil

GROUP	ANIMAL NO.	DAYS ON DIET	BODY WEIGHT				RESULT	SKELETAL MUSCLE DYSTROPHY	OXYGEN CONSUMPTION C.MM./GM./MIN.	MUSCLE CREATINE[1] MG./100 GM.		
			Initial	Days to maximum	Maximum	Final				Sample	1	2
			gm.		gm.	gm.						
A-3 (guinea pig) (3% CSO)	169 ♂	14	264	5	272	268	Normal, killed	—	1.02	Mixed	540	
	168 ♂	22	266	13	318	287	Normal, killed	—	1.53	Mixed	475	441
	167 ♂	35	314	35	375	375	Normal, killed	±	1.62	Mixed	412	408
	170 ♀	57					Normal, biopsy	±	0.62			
	170 ♀	99					P±,[2] biopsy	+++	5.93			
	170 ♀	150					P+, biopsy	++	3.34			
	170 ♀	175	283	163	540	460	P++, killed	++++	2.48	Mixed	131	127
	166 ♀	56					P—, biopsy	±	1.75			
	166 ♀	82					P±, biopsy	+	1.86			
	166 ♀	153					P+, biopsy	±	1.49	Red muscle	296	
	166 ♀	191	280	191	520	520	P+, killed	+ to ++++	3.36	White muscle	212	
A-3 (guinea pig) (6% CSO)	206 ♂	35	272	29	400	339	P++++, killed	++++				
	207 ♂	55	256	53	318	314	P+++, killed	++++				
	208 ♂	38	326	11	404	329	Moribund, killed	++++				
	209 ♀	45	312	6	324	205	P++, killed	++++				
	210 ♀	46	348	34	360	296	P+, killed	++++				
A-4 (rabbit) (4% CSO)	19 ♂	0					Normal, biopsy	—	1.31			
	19 ♂	17					P—, biopsy	—	1.47	Red muscle	284	
	19 ♂	24	950	14	1010	830	P+, killed	++++	2.96	White muscle	310	
	3 ♂	0					Normal, biopsy	—	1.0			
	3 ♂	17					P—, biopsy	—	0.88			
	3 ♂	33					P++, biopsy	++	2.13	Red muscle	305	
	3 ♂	35	1220	27	1500	1050	P++, killed	++++	2.41	White muscle	331	
	13 ♀	0					Normal, biopsy	—	0.50			
	13 ♀	17					P—, biopsy	—	2.38			
	13 ♀	33					P—, biopsy	++	1.95			
	13 ♀	54					P+, biopsy	+++	3.84	Red muscle	154	
	13 ♀	75	1370	57	1520	1225	P++++, killed	++++	4.55	White muscle	136	
	18 ♂	0					Normal, biopsy	—	0.71			
	18 ♂	16					P—, biopsy	±	1.47			
	18 ♂	53					P+, biopsy	+	1.91			
	18 ♂	105	1135	89	1910	1330	P++++, killed	++ to ++++	2.74	Mixed	306	
A-4 (rabbit) (8% CSO)	26 ♀	48	670	23	890	570	P++++, killed	++++				
	27 ♀	58	950	33	1200	1190	P+, killed	++++				

[1] Creatine determinations were made only on muscles at death of animal.

[2] Symptoms of paralysis the extent of which is indicated by plus signs.

TABLE 4

Summary of results with guinea pigs and rabbits fed the synthetic diet containing lard or cottonseed oil supplemented with an A-D concentrate

GROUP	ANIMAL NO.	DAYS ON DIET	BODY WEIGHT				RESULT	SKELETAL MUSCLE DYSTROPHY	OXYGEN CONSUMPTION C.MM./GM./MIN.	MUSCLE CREATINE[1] MG./100 GM.		
			Initial	Days to maximum	Maximum	Final				Sample	1	2
			gm.		*gm.*	*gm.*						
B-1 (rabbit)	16 ♂	0					Normal, biopsy	—	0.64			
	16 ♂	15					P—,[2] biopsy	+	1.04			
	16 ♂	38	1340	26	1360	1010	P++++ killed	++++	4.04			
	20 ♂	0					Normal, biopsy	—	1.40			
	20 ♂	17					P—, biopsy	+	1.54	Red muscle	220	
	20 ♂	47	1015	28	1270	1050	P++++ killed	++++	4.05	White muscle	201	
B-2 (guinea pig)	171 ♂	14	213	14	234	234	Normal, killed	—	1.46	Mixed	413	444
	192 ♂	25	268	12	312	200	Died, diarrhea	—		Mixed	463	455
	174 ♀	36	251	32	360	339	Normal, killed	—	1.02	Mixed	330	359
	191 ♂	57					Normal, biopsy	—	0.48			
	191 ♂	99					P—, biopsy	±	2.39			
	191 ♂	149					P—, biopsy	±	3.28			
	191 ♂♀	173	250	165	620	500	Moribund, killed	+		Mixed	468	
	176 ♀	56					Normal, biopsy	—	0.54			
	176 ♀	81					Normal, biopsy	—	1.17			
	176 ♀	152					Normal, biopsy	—	1.29	Red muscle	377	
	176 ♀	187	254	187	550	550	Normal, killed	—	2.89	White muscle	449	
	211 ♂	49	356	1	356	238	Moribund, killed	—				
	212 ♀	62	240	60	434	434	Normal, killed	—				
	213 ♂	5	296	1	304	254	Moribund, killed	—				
	214 ♀	57	294	18	335	324	Normal, killed	—				
	215 ♂	62	370	61	446	446	Normal, killed	—				
B-3 (rabbit)	17 ♂	0					Normal, biopsy	—	0.98			
	17 ♂	15					Normal, biopsy	—	1.54			
	17 ♂	32	1300	20	1580	1120	P—, died	—				
	7 ♂	0					Normal, biopsy	—	1.12			
	7 ♂	17					Normal, biopsy	—	0.68			
	7 ♂	47					Normal, biopsy	—	1.61	Red muscle	410	
	7 ♂	57	1000	46	1200	970	P—, died	—		White muscle	616	
	28 ♀	50	720	48	1320	1040	P—, died	+				
	29 ♀	51	520	48	830	800	P—, died	±				
	30 ♀	61	880	59	1200	1200	Normal, killed	+				
	31 ♀	62	700	45	1200	1080	Normal, killed	+				

[1] Creatine determinations were made only on muscles at death of animal.
[2] Symptoms of paralysis the extent of which is indicated by plus signs.

lesions and these consisted of scattered, atypical, necrotic fibers. This animal was killed when moribund, and at autopsy was found to have an extensive necrotizing bronchopneumonia. The creatine content of its muscle was within the normal range; however, the oxygen consumption was higher than normal on a previous biopsy. Four of the six rabbits in group B-3 died after an acute attack of diarrhea, the other two rabbits were killed after being on the diet for about 2 months. No gross symptoms of paralysis developed in these animals. At autopsy the muscles of these rabbits were apparently normal in color and consistency; however, on microscopical examination three of the rabbits were found to have several scattered necrotic muscle fibers of recent development. The growth and condition of the muscles of these animals was in marked contrast to the pale, streaked, atrophic and markedly degenerate muscles of the rabbits which were fed the basal synthetic diet supplemented with cod liver oil and had died of muscle dystrophy.

Three out of five of the guinea pigs and all of the rabbits fed the basal synthetic diet plus A-D concentrate without added fat developed characteristic muscle lesions as seen in table 5 (group C-1). However, the survival and growth of these animals was improved as compared to those fed the lard-cod liver oil rations in group A. In both groups of guinea pigs (C-2, C-3) in which the saponifiable fraction of cod liver oil and of cottonseed oil furnished the source of fatty acids a degeneration of their skeletal muscles also resulted.

As seen in table 6 all of the guinea pigs of group D-1 which received the supplement of cod liver oil, except the animal that was killed after 11 days on the ration developed some degree of muscle degeneration. In contrast only one of the five guinea pigs of group D-2 which was given a supplement of cod liver oil concentrate in cottonseed oil solution developed slight muscle changes during the course of the experiment.

The results obtained with guinea pigs fed the grain diet with and without the cod liver oil supplement are also given

in table 6. Guinea pigs nos. 186 to 190, inclusive, were started on experiment in October, 1934, while animals nos. 196 to 205 were placed on experiment in July, 1935. None of the first series of animals on the grain diet developed muscle dystrophy. However, two of the animals developed a type of flaccid paresis which was different from the stiffness or paralysis accompanying advanced muscular dystrophy. Several of the

TABLE 5

Summary of results with guinea pigs fed the synthetic diet without added fat, and the synthetic diet containing the saponifiable fractions of cod liver oil and cottonseed oil supplemented with an A-D concentrate

GROUP	ANIMAL NO.	DAYS ON DIET	BODY WEIGHT				RESULT		SKELETAL MUSCLE DYSTROPHY
			Initial	Days to maximum	Maximum	Final			
			gm.		gm.	gm.			
C-1 (guinea pig)	226 ♂	67	354	56	492	490	P±,	killed	+ to ++++
	227 ♂	66	300	56	384	352	P—,	killed	—
	228 ♂	67	316	40	444	410	P—,	killed	—
	229 ♂	61	358	56	452	411	P—,	killed	+
	230 ♂	43	330	35	400	355	P—,	died	++
C-1 (rabbit)	32 ♀	56	750	29	1050	980	P±,	killed	+++
	33 ♀	61	720	61	1050	1050	P±,	killed	++ to ++++
	34 ♀	62	770	59	1240	1210	P±,	killed	++ to ++++
C-2 (guinea pig)	216 ♀	62	300	62	454	454	P—,	killed	+
	217 ♂	47	380	41	410	336	P++++,	killed	++++
	218 ♀	55	296	50	364	302	P++++,	killed	++++
	219 ♀	63	336	43	433	420	P—,	killed	+
	220 ♂	56	312	41	464	409	P++++,	killed	++++
C-3 (guinea pig)	221 ♂	61	308	15	385	290	P++,	killed	++++
	222 ♀	38	289	25	297	214	P++,	died	++ to +++
	223 ♂	63	284	34	374	296	P—,	killed	+ to ++
	224 ♂	53	308	34	438	311	P+++,	died	++++
	225 ♂	57	314	34	399	349	P—,	killed	++

animals developed generalized edema and ascites which may have been due to the so-called nutritional edema resulting from the low intake of vegetable proteins.

Two of the animals fed the grain ration in the second series showed characteristic muscle changes. These results are confusing; however, when the data for group E-2 are considered it seems apparent that the addition of cod liver oil to the grain diet did increase the rate of development and severity of the

TABLE 6

Summary of results on guinea pigs fed modified diet 11 supplemented with cod liver oil and A-D concentrate and animals fed grains and grains plus cod liver oil

GROUP	ANIMAL NO.	DAYS ON DIET	BODY WEIGHT				RESULT		MUSCLE DYSTROPHY	OXYGEN CONSUMPTION C.MM./GM./MIN.	MUSCLE CREATINE[1] MG./100 GM.		
			Initial	Days on maximum	Maximum	Final					Sample	1	2
			gm.		gm.	gm.							
D-1	179 ♂	11	222	11	245	245	Killed,	normal	—	1.67	Mixed	478	497
	182 ♀	34	238	12	304	283	Killed,	weak	+ to ++	3.90	Mixed	320	370
	178 ♂	42	275	42	355	355	Killed,	diarrhea	++	1.80	Mixed	349	335
	175 ♀	59	218	42	355	284	Killed,	P—[2]	++++	3.38	Gastrocnemious	197	262
	177 ♂	78					Biopsy		+	2.35			
	177 ♂	112					Biopsy		±	0.57			
	177 ♂	150					Biopsy		±	2.83			
	177 ♂	206	266	206	750	750	Killed		+	2.81	Mixed	358	
D-2	185 ♀	12	272	12	299	299	Killed,	normal	—	1.11	Mixed	492	512
	183 ♀	23	204	10	249	200	Died,	diarrhea	—		Quadriceps	375	
	181 ♂	28	261	22	359	288	Killed,	diarrhea	—	1.39	Mixed	437	416
	180 ♂	80					Biopsy		—	0.43			
	180 ♂	112					Biopsy		—	0.65			
	180 ♂	148					Biopsy		—	0.61			
	180 ♂	199	258	194	920	920	Killed,	normal	—	2.05	Mixed	448	
	184 ♀	83					Biopsy		—	0.91			
	184 ♀	112					Biopsy		—	1.04			
	184 ♀	148					Biopsy		—	1.18			
	184 ♀	199	286	194	800	760	Normal,	killed	+	2.75	Mixed	490	
E-1 grains	187 ♀	7	232	1	245	235	Normal,	killed	—	0.96	Gluteus	469	
	189 ♂	21	223	12	258	216	Normal,	killed	—	1.35	Mixed	498	528
	188 ♀	28	220	22	280	253	Killed,	paresis	—	1.37	Mixed	423	415
	190 ♂	31	232	28	298	276	Died,	paresis	—		Mixed	368	388
	186 ♂	50	216	7	222	200	Emaciated,	killed	—	1.47	Triceps	486	
	196 ♀	52	315	34	326	214	P—,	died	++				
	197 ♀	40	216	13	240	194	P-moribund,	killed	—				
	198 ♂	54	234	13	246	205	P—,	died	—				
	199 ♂	51	232	45	273	210	P-moribund,	killed	++++				
	200 ♂	51	272	38	300	265	P-moribund,	killed	—				
E-2 grains CLO	201 ♀	39	230	20	285	202	P+++,	died	++++				
	202 ♂	47	210	38	236	190	P++,	died	++++				
	203 ♂	23	272	22	292	282	P—,	died	++++				
	204 ♂	47	232	36	234	180	P—,	died	++++				
	205 ♀	41	240	29	288	245	P++moribund,	killed	++++				

[1] Creatine determinations were made only on muscles at death of animal.

[2] Symptoms of paralysis the extent of which is indicated by plus signs.

muscle lesions during the experimental period. Growth and survival were very poor on both rations as would be expected from its numerous deficiencies. A daily supplement of 7 to 10 cc. of tomato juice furnished the antiscorbutic vitamin and the cod liver oil was fed emulsified in the tomato juice-yeast mixture.

The individual values obtained for oxygen consumption and creatine content of the excised dystrophic and normal skeletal muscles which are given in tables 2, 3, 4 and 6 are summarized in table 7.

TABLE 7

Significance of relations between the extent of degeneration, oxygen consumption and creatine content of the excised muscle tissue

OBSERVATION	ANIMAL	EXTENT OF DEGENERATION	NUMBER OF OBSERVATIONS	MEAN		COEFFICIENT OF VARIABILITY
				Value	Standard deviation	
				c.mm./gm./min.		
Oxygen consumption of muscles	Guinea pig	—	31	1.27 ± 0.08	0.67	52
		±, +, ++	16	2.24 ± 0.16	0.93	42
		+++, ++++	7	3.68 ± 0.47	1.85	50
	Rabbit	—	21	1.20 ± 0.08	0.52	43
		±, +, ++	7	1.72 ± 0.10	0.39	23
		+++, ++++	9	3.23 ± 0.22	0.97	30
				mg./100 gm.		
Creatine content of muscles	Guinea pig (mixed sample)	—	35	442 ± 9.4	83	19
		±, +	5	427 ± 15.8	52	12
		++	8	366 ± 8.5	36	10
		+++, ++++	11	155 ± 15.4	76	49
	Rabbit (white muscle)	++++	7	248 ± 13.5	53	21
	(red muscle)	Same animals	7	281 ± 22.4	88	31

The oxygen consumption of the dystrophic guinea pig muscle is apparently increased as compared to the average value of 1.27 ± 0.08 c.mm. for the thirty-one determinations on animals maintained on several diets whose muscles failed to show histological evidence of degeneration. The mean value of 2.24 ± 0.16 c.mm. for muscles in the initial stages of degeneration can only be considered as suggestive of an increase, but the mean value of 3.68 ± 0.47 c.mm. for the +++ and ++++ dystrophic muscles appears significantly higher than normal in spite of the fewer determinations and high variability of the data.

Victor ('34) found an increase in the oxygen consumption of dystrophic rabbit muscle and additional data are provided in the present experiments. The average normal oxygen consumption of the excised rabbit muscle in this series was 1.2 ± 0.08 c.mm. as compared to 1.4 c.mm. as obtained by Victor. The mean value of 3.23 ± 0.22 c.mm. for the +++ and ++++ degenerated muscles suggests a significant increase in oxygen consumption with the degeneration, but because of the variability of the data and relatively few determinations the exact magnitude of the increase and its relation to the specific stage of degeneration is not clear. The method of grouping the oxygen consumption values on the basis of + to ++++ degeneration is probably not satisfactory since as yet this method does not take into consideration the type or stage but rather the amount of degeneration as pointed out by Goettsch and Brown ('32).

The rate at which oxygen is consumed by the degenerating muscle usually remains consistently high from the beginning of the experiment and falls only slightly with time, while that of the normal muscle falls rapidly during the first few minutes in the respirometer and then reaches a base level which is fairly constant. The biopsy data on guinea pigs no. 170, table 3, suggests that the oxygen consumption may increase to a maximum, and then decline even though the degeneration and paralysis continues. This may explain the relatively small increase obtained in such cases as guinea pig no. 163 of table 2. The oxygen consumption values on the samples of muscle obtained at biopsy for several other animals show an increase with increasing degeneration. The record of rabbit no. 13 in table 3 and rabbits nos. 16 and 20 in table 4 are of special interest in this regard. The data for other animals show a less marked but often a definite increase with degeneration, while for some animals the result was variable. In contrast to the apparent increase in respiration of dystrophic rabbit and guinea pig muscle Knowlton and Hines ('34) report that there is no change from normal in the oxygen consumption of atrophic gastrocnemii of rats produced by sciatic nerve section.

The data on muscle creatine show that muscle dystrophy in the guinea pig as well as in the rabbit (Goettsch and Brown, '32; Woodward, '32) is accompanied by a loss of creatine from the skeletal muscles which is roughly proportional to the extent of degeneration.

The so-called red muscles, represented for example by the semitendinosus and soleus in the rabbit are normally lower in creatine content than the white muscles, such as the adductors of the thigh, gluteus and quadriceps. Goettsch and Brown ('32) found an average of 314 $\pm$ 7.1 mg. creatine for the red and 463 $\pm$ 5.1 mg. creatine for the white muscles in rabbits. These normal data are used for comparison with the creatine content of muscles from rabbits with ++++ degeneration in the present series. Seven animals had an average creatine of 248 $\pm$ 13.5 mg. for the red and 281 $\pm$ 22.4 mg. creatine for the white muscle samples. This shows a marked decrease from normal and a relatively larger reduction of creatine in the white muscles as compared to the red in the same animal. A basis for this difference in reduction of creatine between red and white muscles is evident on histological examination since the red muscles usually maintain their integrity longer and are involved to a lesser extent in the degenerative process than the white muscles even in advanced stages of dystrophy.

DISCUSSION

The data presented in this paper (groups A-1, A-2) confirm the previous studies of Madsen, McCay and Maynard ('33, '35) in showing that when guinea pigs and rabbits are fed a synthetic diet containing lard as the principal source of fat and cod liver oil as a source of vitamins a severe degeneration of the skeletal muscles results. They also show that the substitution of cottonseed oil for the lard of this diet has probably no influence on the occurrence or severity of the dystrophy, although it may cause a slight improvement as regards growth and survival (groups A-3, A-4). However, the data obtained with this synthetic diet when the cod liver oil was

replaced by a concentrate of it fed in cottonseed oil solution suggest a protective effect of the cottonseed oil against the muscle degeneration (groups B-2, B-3). In the previous studies such a replacement, in which another A-D concentrate was fed in a cottonseed oil solution, markedly delayed the onset of the muscle lesions; but in the present experiment replacement by a concentrate without oil addition did not cause this favorable effect (group B-1). This suggests that the lard also may influence the muscle dystrophy or that the cottonseed oil was definitely protective. The elimination of lard from the diet, however, without the addition of cottonseed oil had little effect in reducing the occurrence of the lesions (group C-1). Thus it is indicated that animal fat per se is not a primary harmful factor. It is evident, however, that the protective effect of cottonseed oil is not effective where cod liver oil is fed instead of the concentrate at the levels used in this experiment (groups A-1 to A-4).

In the previous experiments (Madsen, McCay and Maynard, '35) a synthetic diet containing lard as the source of fat and supplemented with the saponifiable fraction of cod liver oil proved to favor the development of muscle lesions more than when the non-saponifiable portion was added to the diet. Further data show that the synthetic diet without added fat other than the saponifiable fraction of cod liver oil (group C-2) or of cottonseed oil (group C-3) with the concentrate as the source of vitamins produces severe muscle lesions. These experiments furnished little additional information except that no protective effect was obtained from this fraction of cottonseed oil, in contrast to the results with the oil as a whole (groups B-2, B-3).

The comparison with the modified diet 11 of Goettsch and Pappenheimer (groups D-1, D-2) resulted in lesions in all of the guinea pigs that were on the experiment longer than 11 days where cod liver oil was fed and only in a slight degree in one case when the ration was supplemented with a cottonseed oil solution of the concentrate. These results are noteworthy, as they are probably related to those obtained with the synthetic diets previously discussed.

The significant feature of the experiments (groups E-1, E-2) in which the grain diet was fed, was the development of lesions in all cases where cod liver oil was added and in two cases out of ten where the oil was omitted. This shows, in confirmation of the previous studies (Madsen, McCay and Maynard, '35) that cod liver oil is effective in the development of muscle degeneration with diets of natural foods as well as with the synthetic diets, and it is further demonstrated that cod liver oil is not the only factor concerned in this dietary disease. The diet of grains is obviously incomplete in several respects and among its deficiencies other contributory factors may be accounted for. No case of muscle dystrophy has been seen as yet in the control animals (group F-1) fed a stock ration consisting of grains as fed to group E and receiving in addition an abundance of alfalfa and lettuce. The effect of cod liver oil addition to this ration was not studied.

The data obtained on the oxygen consumption of excised dystrophic rabbit and guinea pig muscle are variable and limited but for the most part consistent in showing an increase as compared to normal values. Further work is necessary on this problem to determine the cause of this altered respiration, its relation to the specific stages of degeneration, and its relation to the respiration of other tissues as well as to the metabolism of the animal during life. The lowered creatine content of the degenerating skeletal muscles, the tendency to an increased oxygen consumption and similar histological picture of muscle lesions in rabbits and guinea pigs on the synthetic rations and the modified diet 11 of Goettsch and Pappenheimer, suggests that the disease is the same in both species.

It is recognized that the present experiments still leave much to be explained as to the causes of the dystrophy which is produced in Herbivora by dietary means. It is hoped in further studies to give attention to other components of the diet in addition to continuing studies on the fat interrelationships which have been the primary concern of the present experiment.

SUMMARY

A study is reported of the skeletal muscle dystrophy which occurs in guinea pigs and rabbits fed synthetic diets containing cod liver oil or its concentrate and with lard or cottonseed oil as the chief source of fat. The basal synthetic diet alone without added fat other than the non-saponifiable fraction of cod liver oil as a source of vitamins produced dystrophy to nearly the same extent as the same diet which included 6 per cent of lard; but the use of cottonseed oil in place of the lard resulted in a high degree of protection against the development of muscle lesions. No such protective effect resulted when cod liver oil was fed as the source of vitamins in place of its non-saponifiable fraction.

Experiments with restricted natural food diets demonstrated these relations further and furnished additional evidence that the fat relationships studied are probably not the only factors in the production of muscle lesions. Nutritional muscular dystrophy in the rabbit and guinea pig is similar histopathologically and is characterized by a lowered creatine content and a tendency to an increased oxygen consumption of the excised muscle tissue.

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PLATE 1

EXPLANATION OF FIGURES

1 Rabbit 2. Group A-2. Fourteen days on diet. Section of masseter muscle showing hyaline degeneration of muscle fibers, multiplication of muscle nuclei, infiltration of cells and beginning calcification of necrotic fibers.

2 Rabbit 20. Group B-1. Forty-seven days on diet. Section of gastrocnemius muscle. Note almost complete disappearance of muscle fibers and the unusual picture of a multinucleated atrophic muscle fiber near the bottom of the figure as compared to the nearly normal fiber at the top.

3 Guinea pig 202. Group E-2. Forty-seven days on diet. Section of adductor muscle of the thigh showing massive necrosis of muscle fibers.

4 Guinea pig 170. Group A-3. One hundred and seventy-five days on diet. Cross section of quadriceps muscle showing scattered muscle fibers with replacement of previously degenerated muscle with adipose tissue.

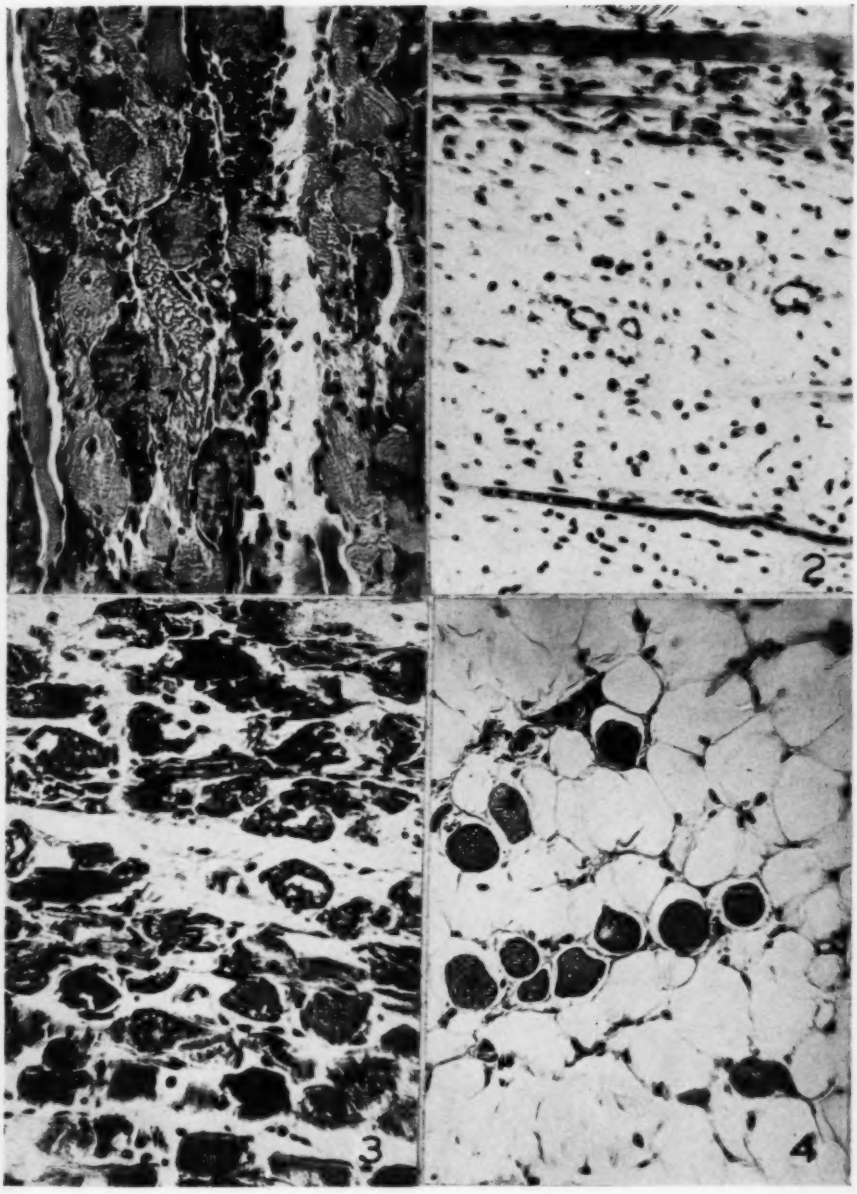
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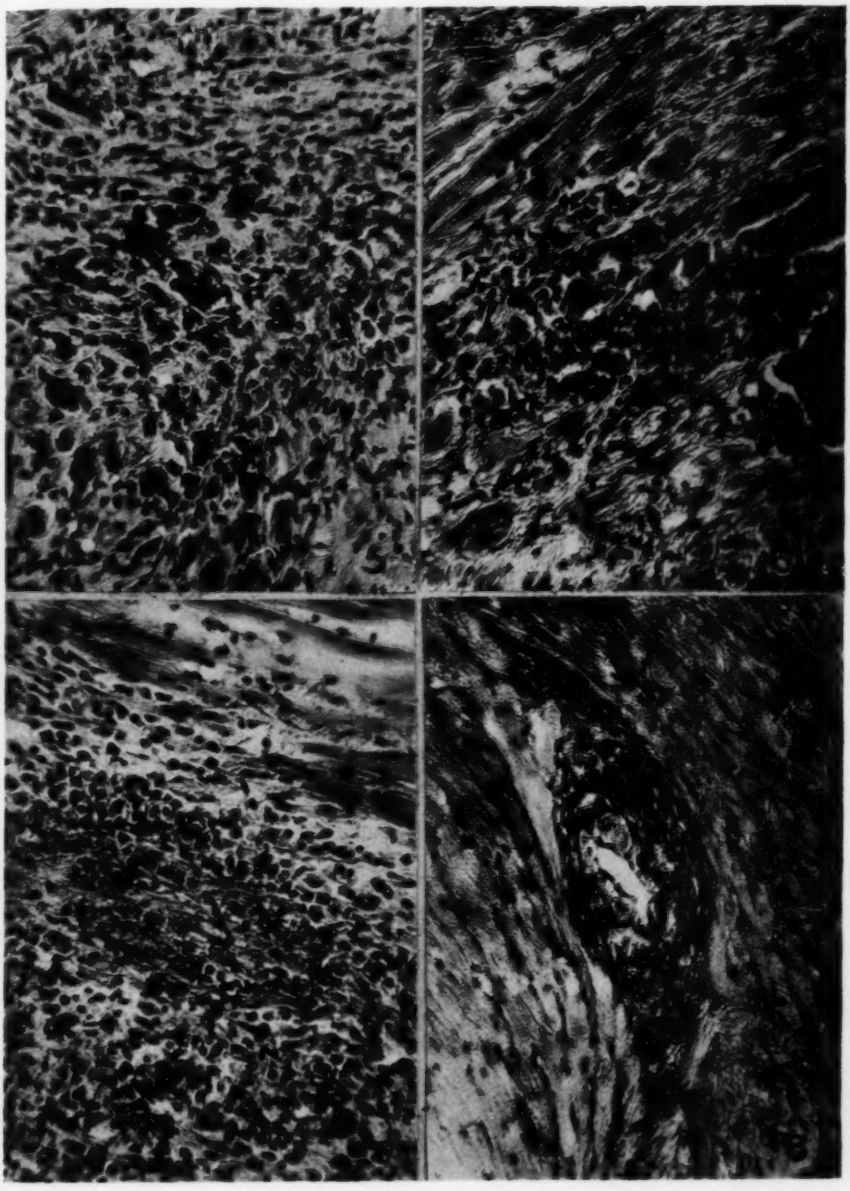
PLATE 2

EXPLANATION OF FIGURES

5 Rabbit 3. Group A-4. Thirty-five days on diet. Section of cardiac muscle with atrophy and calcification of necrotic muscle fibers.

6 Rabbit 14. Group A-2. Forty days on diet. Section of cardiac muscle showing calcifications of necrotic muscle fibers, multiplication of muscle nuclei with some formation of multinucleated plasmatic masses.

7 Rabbit 5. Group A-2. Seventeen days on diet. Section of cardiac muscle. Degeneration of muscle with proliferation of cells about densely eosin staining fibers.

8 Rabbit 2. Group A-2. Fourteen days on diet. Small artery in wall of left ventricle showing intimal thickening and fragmentation of the inner elastic membrane.